2018

CYCLODEXTRIN-PROMOTED DETECTION SYSTEMS FOR BIOLOGICAL MATRICES AND ENVIRONMENTAL APPLICATIONS

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CYCLODEXTRIN-PROMOTED DETECTION SYSTEMS
FOR BIOLOGICAL MATRICES AND ENVIRONMENTAL
APPLICATIONS

BY
DANA J. DISCENZA

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
CHEMISTRY

UNIVERSITY OF RHODE ISLAND

2018

DOCTOR OF PHILOSOPHY DISSERTATION
OF
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2018

ABSTRACT

The detection of persistent organic pollutants (POPs) in complex biological and environmental samples is an important research priority due to the known persistence, toxicity, and carcinogenicity of these compounds. Some examples of POPs include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, alcohols and phthalates. Human exposure to these compounds has been well-documented in the scientific literature, and can occur through a variety of mechanisms, such as through an individual's exposure to contaminated air and soil or through the ingestion of contaminated food or water. Additionally, individuals who have been exposed to POPs contain small amounts of these compounds in their blood, breast milk, and urine.

Currently used methods for the detection of POPs rely heavily on mass spectrometry-based techniques. These methods are known to be highly sensitive; however, they are not ideal for rapid detection applications for several reasons, including: (a) Mass spectrometry-based techniques are time-consuming, due to the significant time spent performing method development and possible need for extraction of compounds from aqueous media prior to analysis. These instruments are also extremely expensive and require significant user expertise. Due to these drawbacks, there is a need for a practical, rapid, sensitive, and selective method for the detection of persistent organic pollutants in complex biological and environmental matrices, especially one that does not rely on mass spectrometry-based techniques.

Previous work in our group focused on addressing the problem of POP detection in complex environments through the use of cyclodextrin-promoted energy transfer for

the detection of photophysically active, aromatic toxicants. This system relies on the ability of cyclodextrin to act as a supramolecular scaffold and promote proximity-induced energy transfer from an aromatic energy donor to a high quantum yield fluorophore acceptor. These interactions lead to a bright, turn-on fluorescence signal that is unique for each cyclodextrin-fluorophore-toxicant combination. These fluorescence signals can be used to develop array-based detection systems, where each cyclodextrin-fluorophore-toxicant combination results in a unique signal; by using a variety of different cyclodextrins and/or fluorophores, a unique identifier for each toxicant can be generated. Our group has published a number of articles highlighting the advantages of cyclodextrin-promoted energy transfer for the detection of persistent organic pollutants. We have shown that this method operates successfully in purified buffer systems, that it can form a part of an oil spill remediation strategy using cyclodextrin-promoted toxicant extraction following by fluorescence detection, and that it can be used for the detection of a wide range polar and nonpolar, photophysically active, aromatic toxicants.

While the use of cyclodextrin-promoted energy transfer has been well-documented by our group, it is only effective for the rapid, sensitive, and selective detection of photophysically active, aromatic toxicants. Recent events involve environmental contamination with non-photophysically active toxicants, including the 2014 chemical spill in Elk River, West Virginia and a 2015 tanker truck crash in Bourne, Massachusetts. In response to these recent events, our group has developed a new detection method we have termed cyclodextrin-promoted fluorescence modulation. This method relies on the ability of cyclodextrin to promote proximity-induced non-covalent

interactions between a high quantum yield fluorophore and a non-photophysically active toxicant. These interactions, in turn, lead to a proximity-induced change in the fluorescence signal of the high quantum yield fluorophore and are highly combination specific for each cyclodextrin-fluorophore-toxicant. These fluorescence changes can be subjected to array-based statistical analyses to develop extremely high analyte selectivity while maintaining sensitivity. The work reported in this dissertation is divided into two objectives: (1) Development of a cyclodextrin-based detection system for non-photophysically active toxicants; and (2) Application of cyclodextrin-promoted fluorescence modulation for toxicant detection in complex biological and environmental samples.

The first manuscript, “Sensitive and Selective Detection of Alcohols via Fluorescence Modulation,” describes the detection of aliphatic alcohols using cyclodextrin-promoted, proximity-induced fluorescence modulation of a high-quantum yield fluorophore in the aftermath of a January 2014 chemical spill in Elk River, West Virginia. Fluorescence modulation occurred when the analyte was held in close proximity to the fluorophore via non-covalent cyclodextrin-analyte-fluorophore interactions. These interactions led to unique modulation responses for each combination investigated. This method was highly sensitive (low micromolar detection limits) and selective (99% differentiation between structurally similar analytes). This work was the first report of this fundamentally new detection approach for alcohols. This manuscript was published in *Supramolecular Chemistry* in 2016 and was awarded the Sessler Early Career Research Prize in 2018.

The second manuscript, “Selective Detection of Non-Aromatic Pesticides via Cyclodextrin-Promoted Fluorescence Modulation,” describes the application of cyclodextrin-promoted fluorescence modulation to another class of analytes, aliphatic organochlorine pesticides. These analytes were detectable through quantifying proximity-induced changes in the fluorescence emission spectrum of a high quantum yield fluorophore upon introduction of the analyte. This work resulted in detection limits as low as 5.2 μM and 100% selectivity in array-based identification. This manuscript was published in *New Journal of Chemistry* in 2016.

The third manuscript, “Detection of Organochlorine Pesticides in Contaminated Marine Environments via Cyclodextrin-Promoted Fluorescence Modulation,” describes the rapid, sensitive, and selective detection of four previously investigated organochlorine pesticides in a variety of water sources across the state of Rhode Island using cyclodextrin-promoted fluorescence modulation. The research reported in this manuscript builds on previously reported work on the detection of organochlorine pesticides in purified buffer systems (Manuscript 2) and introduces complex matrix effects associated with marine environments. This work reported high sensitivity (low micromolar detection limits), high sensitivity (100% differentiation between structurally similar analytes), and general applicability (for different water samples with varying salinity and pH as well as for different water temperatures). This manuscript was published in *ACS Omega* in 2017.

The fourth manuscript, “Detection of Benzene and Alkylated Benzene Derivatives in Fuel Contaminated Environments,” describes the detection of fuel components such as benzene, toluene, and xylenes using cyclodextrin-promoted

fluorescence modulation. This method was applied to the detection of fuel components in purified buffer systems as well as in contaminated aqueous environments following a March 2015 tanker truck crash in the Bourne Rotary in Bourne, Massachusetts. This detection scheme was highly sensitive and selective, and showed well-separated signals for structural isomers of xylene (*ortho*, *meta*, and *para*) which is hard to do without specialized mass spectrometry-based techniques. This manuscript was published in *Clean – Soil, Air, Water* in 2016.

The fifth manuscript, “Efficient Detection of Phthalate Esters in Human Saliva via Fluorescence Spectroscopy,” describes the application of cyclodextrin-promoted fluorescence modulation to another class of compounds (phthalate esters) and to a complex, biological fluid (human saliva). Saliva contains enzymes that can break down γ -cyclodextrin, so α -cyclodextrin and β -cyclodextrin were used as supramolecular hosts for this work. This work was highly selective and was the first report from our group on the use of array-based analysis to differentiate between various concentrations of a single analyte. This manuscript was published in *Analytical Letters* in 2018.

The sixth manuscript, “Detection of Bisphenol A and Derivatives in Human Urine via Cyclodextrin-Promoted Fluorescence Modulation,” describes the detection of Bisphenol A (BPA) and structurally-similar BPA derivatives in purified buffer and human urine using cyclodextrin-promoted fluorescence modulation. Bisphenols have been used as plasticizers and have been found in the biological fluids of people who have come into contact with these compounds. These compounds are well-documented endocrine disruptors; therefore, their detection is of high priority. This work uses only γ -cyclodextrin to promote fluorescence modulation of three high quantum yield

fluorophores: BODIPY-Me, BODIPY-Ph, and Coumarin 6. Unique changes in fluorescence were seen for each cyclodextrin-analyte-fluorophore combination investigated. These results translated to 100% differentiation between structurally similar bisphenol compounds in both purified buffer and human urine environments. This manuscript was published in *Analytical Methods* in 2018.

The work included in this doctoral dissertation provides crucial information, demonstrating the scope of cyclodextrin-promoted fluorescence modulation. This information will be used in the future development of solid-state devices for the rapid, sensitive, and selective detection of persistent organic pollutants in a wide range of biological matrices and for a variety of environmental applications.

ACKNOWLEDGMENTS

“I want to say to all the young women out there: There are going to be people along the way who will try to undercut your success or take credit for your accomplishments or your fame. But if you just focus on the work and you don’t let those people sidetrack you, someday when you get where you’re going, you’ll look around and you will know – that it was you, and the people who love you, who put you there. And that will be the greatest feeling in the world. Thank you for this moment.”

-Taylor Swift (2016 Grammy Awards)

Four years later, earning my PhD in Chemistry is truly the greatest feeling in the world. To everyone who has been there for me, loved me, and supported me over the past four years – thank you for this moment – this is for you.

First and foremost, I would like to thank my graduate advisor, Professor Mindy Levine, for all of the support, encouragement, and fun over the past few years. I joined your research group back in 2014 because of your passion for research and problem-solving. I knew right away you were the perfect advisor for me, and joining your group was hands-down the best decision. You have been the most incredible mentor, and I owe much of my professional success to you. Thank you for always encouraging me to be strong and strong-willed in both research and in life. I will sincerely miss all of your antics and all of our fun times and crazy conversations. You are a total boss, Boss! Thank you for everything.

I would also like to thank my committee: Professor Brenton DeBoef, Professor Arijit Bose, and Professor Geoff Bothun, for your insight and support throughout my graduate career. I have learned so much from each of you over the years, and I am so very grateful for your science advice and constructive criticism.

Thank you so much to my parents, Barbara DiScenza and Ronald DiScenza, MD, for being the absolute best parents and always supporting my curiosity and love of science no matter what. You are the reason I decided to pursue science in the first place.

You two are the smartest, strongest, most wonderful people in my life. I wouldn't have been able to do any of this without your support. I hope I have made the two of you proud. I love you both more than life itself! Thank you so much for everything.

Thank you to my amazing siblings: Alexa, Dylan, and Andrew for all of the advice and the much-needed comic relief over the years! You are all so smart and phenomal in your own ways, and you three are truly my inspiration for getting through school. Thank you for always being there for me and building me up every step of the way. I love you guys (and Caesar and Daisy too, of course).

I would also like to acknowledge the rest of my loving family. To my grandparents, Arnold and Gloria Schmiedel and Anthony and Justine DiScenza – I love and miss each of you so very much.

I would like to thank my boyfriend, Captain Scott Karas, for all of the love and support during graduate school. Meeting you in the hallway of Pastore with your typical, “Happy Monday,” was one of the highlights of my time at URI. Thank you for always encouraging me to be positive and live life to the fullest. I just love you more than words could ever describe. I am so excited to start the next chapter of our life together!

A huge thank you to all of the middle school, high school, undergraduate, and graduate students that I have had the opportunity to mentor over the years. The work reported in this dissertation would not have been possible without your hard work and dedication. You are all so outstanding, and I hope your research experience in our group was a positive one!

Lastly, thank you to all current and former members of the Levine Group and all of my friends in the Chemistry Department at the University of Rhode Island. I have

had the most incredible graduate school experience. Thank you for all of the fun times and science advice. Continue “thinking big,” and using your research to make the world a better place!

DEDICATION

I wish to dedicate this doctoral dissertation to my mother, Barbara DiScenza. Thank you for always encouraging me to reach for the stars and for supporting me in everything I have ever wanted to do. I love you so much!

PREFACE

The dissertation of my research has been presented in manuscript format according to the guidelines of the graduate school of the University of Rhode Island. The complete dissertation is divided into six manuscripts. The first manuscript (Chapter 1) was published in *Supramolecular Chemistry* in 2016 with authors Dana J. DiScenza and Mindy Levine. The second manuscript (Chapter 2) was published in *New Journal of Chemistry* in 2016 with authors Dana J. DiScenza and Mindy Levine. The third manuscript (Chapter 3) was published in *ACS Omega* in 2017 with authors Dana J. DiScenza, Julie Lynch, Jasmine Miller, Molly Verderame, and Mindy Levine. The fourth manuscript (Chapter 4) was published in *CLEAN – Soil, Air, Water* in 2016 with authors Dana J. DiScenza, Molly Verderame, and Mindy Levine. The fifth manuscript (Chapter 5) was published in *Analytical Letters* in 2018 with authors Dana J. DiScenza, Melissa A. Smith, and Mindy Levine. The sixth manuscript (Chapter 6) was published in 2018 in *Analytical Methods* with authors Dana J. DiScenza, Julie Lynch, Ezra Feder, and Mindy Levine.

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CHAPTER 1

Published in *Supramolecular Chemistry* **2016**, 28, 881-891

Sensitive and Selective Detection of Alcohols via Fluorescence Modulation

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Manuscript 1

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ABSTRACT

Reported herein is the selective detection of aliphatic alcohols using cyclodextrin-promoted proximity-induced fluorescence modulation of a high-quantum yield fluorophore. This fluorescence modulation occurred when the analyte was held in close proximity to the fluorophore via non-covalent cyclodextrin-analyte-fluorophore interactions, and led to unique modulation responses for each analyte, fluorophore, and cyclodextrin investigated. These changes in fluorescence were used for the generation of an array using linear discriminant analysis that successfully generated unique pattern identifiers for 99% of the investigated analytes, and could detect alcohols at micromolar concentrations. These results represent a fundamentally new detection approach for these challenging analytes, and have significant potential in the development of novel detection schemes.

INTRODUCTION

The sensitive and selective detection of alcohols is an important research objective with implications in public health and environmental remediation, as many of these alcohols are known or suspected toxicants.¹⁻³ Selectivity in distinguishing aliphatic alcohols from similar analogues is particularly crucial, as structurally similar analogues often have widely disparate toxicities. These aliphatic alcohols are found in fuels and fuel additives,⁴ foods and beverages,⁵ and other commercial products.^{6,7} Current methods for aliphatic alcohol detection overwhelmingly rely on mass spectrometry;^{8,9} however, the high sensitivity associated with mass spectrometry often

leads to extraneous signals, which increases the analysis time and limits the ability to conduct high-throughput assays.^{10,11} Some examples of non-mass spectrometry detection methods have been reported, most of which require a derivatization step to enable accurate detection.^{12,13}

A January 2014 chemical spill in Elk River, West Virginia of more than 7500 gallons of 4-methylcyclohexanemethanol (4-MCHM)^{14,15} highlighted a key problem with the use of mass spectrometry-based detection in complex environments: the need for *a priori* knowledge of the toxicant of interest to enable high-throughput detection of contaminated samples.^{16,17} Because every compound generates a mass spectral signal, first responders needed to know the identity of the contaminant prior to detection and clean up. In the case of the Elk River spill, this knowledge was not disclosed by the offending company in a timely fashion, significantly hampering remediation efforts.^{18,19} We recently developed a fundamentally new approach for small-molecule detection using γ -cyclodextrin as a supramolecular scaffold to promote proximity-induced energy transfer from aromatic toxicants and toxicant metabolites to high-quantum yield fluorophores (Figure 1).²⁰⁻²³

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

This energy transfer operates successfully in multiple complex environments, including biological fluids²⁴ and aqueous extracts from crude oil samples,²⁵⁻²⁷ and has been used for the selective array-based detection of 30 aromatic analytes with 99% accuracy in buffer and 92% accuracy in human urine.²⁸

All previous work on cyclodextrin-based detection relied on the ability of the toxicants to act as energy donors to high quantum yield fluorophores,^{29,30} which does not allow for the detection of non-aromatic small molecule analytes such as aliphatic alcohols. Although these alcohols do not participate in proximity-induced energy transfer, they nonetheless are known to bind in or near the cyclodextrin cavity.³¹ This binding, in turn, can lead to significant fluorescence modulation of a high-quantum yield fluorophore held in close proximity by the cyclodextrin scaffold (Figure 2).

Figure 2. Illustration of cyclodextrin promoted fluorescence modulation for aliphatic alcohol detection; dashed lines in the chemical structure indicate some of the possible intermolecular interactions

This modulation relies on favorable intermolecular interactions between the analyte, fluorophore and cyclodextrin, including hydrogen bonding, hydrophobic interactions, CH- π interactions and a variety of other intermolecular forces.³²⁻³⁴ Although all of these forces are well-known in the chemical literature, to the best of our knowledge, they have not been applied to the detection of this highly relevant class of analytes to date. Reported herein is the successful realization of such proximity-induced,

cyclodextrin-promoted fluorescence modulation that uses a multitude of inter-molecular forces to achieve the sensitive and selective detection of a variety of alcohols, including aliphatic, aromatic and benzylic alcohols.

EXPERIMENTAL

Materials and Methods

All analytes (except for compound **8**) and cyclodextrins were obtained from Sigma-Aldrich chemical company. MCHM **8** was obtained from Tokyo Chemical Company as a mixture of *cis*- and *trans*- isomers. Fluorophore **14** was synthesized following literature procedures.³⁵ All fluorescence measurements were performed using a Shimadzu RF 5301 spectrophotometer. Both the excitation slit width and the emission slit width were 1.5 nm. All fluorescence spectra were integrated vs. wavenumber on the *X*-axis using OriginPro software. All arrays were generated using SYSTAT Version 13.

Experimental Details for Fluorescence Modulation Experiments

In a quartz cuvette, 2.5 mL of a cyclodextrin solution and 100 μ L of a fluorophore **14** solution (0.1 mg/mL) were combined. The solution was excited at 460 nm, which is the excitation wavelength to directly excite **14**. About 20 μ L of an analyte solution (1 mg/mL) was added to the cuvette, and fluorescence measurements were repeated at 460 nm. The experiments were repeated for fluorophore **15** and **16** at excitation wavelengths of 420 nm and 490 nm, respectively. The fluorescence spectra were integrated vs. wavenumber on the *X*-axis, and fluorescence modulation was determined using Equation (1).

$$\text{Fluorophore Ratio} = (Fl_{\text{analyte}})/Fl_{\text{blank}} \quad (1)$$

Where Fl_{analyte} is the fluorescence emission of the fluorophore in the presence

of the alcohol, and FI_{blank} is the fluorescence emission of the fluorophore in the absence of the alcohol. Each experiment was repeated four times. The reported values are the average of the results.

Experimental Details for Array Generation Experiments

Array analysis was performed using SYSTAT 13 statistical computing software with the following settings: (a) Classical Discriminant Analysis; (b) Grouping Variable: Analytes; (c) Predictors: Bodipy, Rhodamine 6G, Coumarin 6; (d) Long-Range Statistics: Mahal

Experimental Details for Limit of Detection Experiments

These experiments were conducted following literature-reported procedures,³⁶ to determine the limit of detection (LOD), defined as the lowest concentration of analyte at which a signal can be detected, and the limit of quantification, defined as the lowest concentration of analyte that can be accurately quantified.

RESULTS AND DISCUSSION

Selection of Alcohols

Chart 1. Structures of alcohol analytes **1-5**, **8-12**; control analytes **6**, **7** and **13**; and fluorophores **14-16**

The alcohol analytes were selected to include a wide range of analytes (Chart 1), including those that are structurally similar but have widely disparate toxicities. Ethylene glycol **5** was chosen due to its widespread availability and known toxicity,³⁷ as well as the desire to assay the ability of the cyclodextrin-based system to identify diol motifs via fluorescence modulation. 1-Hexanol (compound **1**) and cyclohexanol (compound **2**) were selected to challenge the ability of the fluorescence modulation system to distinguish between analytes with identical molecular weights, which are difficult to distinguish via high-throughput mass spectral analysis (although such compounds have unique fragmentation patterns in the mass spectrometer).³⁸ 4-MCHM (compound **8**) was selected due to its prevalence in the 2014 Elk River contamination event,¹⁴ as well as its structural similarity to cyclohexanol **2**. Alcohols **9–12** were chosen to assay the ability of the fluorescence modulation technique to detect propargylic alcohol, aromatic alcohols and highly hindered tertiary alcohols, and to distinguish between these analytes and simple primary aliphatic alcohols **1**, **3** and **4**. Control analytes **6** and **7**, which lack free hydroxyl moieties, were also included in these investigations, as was tetrahydrofuran (**13**), the solvent used to dissolve the fluorophores.

Selection of Fluorophores

These compounds include three of the major classes of high-quantum yield organic fluorophores: BODIPY, Rhodamine, and Coumarin. They were either commercially available or were synthesized following literature-reported procedures.³⁵ The efficacy of these fluorophores in energy transfer-based detection systems has already been established by us²⁰ and others.³⁹⁻⁴¹

Selection of Cyclodextrins

Cyclodextrins were chosen as supramolecular hosts for this proximity-induced fluorescence modulation, as a result of their high aqueous solubility and known ability to promote non-covalent intermolecular interactions.⁴² Most of these interactions rely on the ability of cyclodextrin to bind hydrophobic guests in the hydrophobic interior cavity;⁴³ an alternate mode of complexation involves hydrogen bonding between the cyclodextrin hydroxyl groups (hydrogen bond donors) and hydrogen bond acceptor moieties on small molecule guests.⁴⁴ The alcohol analytes are expected to associate through hydrogen bond formation involving the free hydroxyl groups, as well as hydrophobically driven inclusion involving the hydrocarbon segments of the alcohol structures. We selected four commercially available cyclodextrin hosts: α -cyclodextrin (α -CD), β -cyclodextrin (β -CD), randomly methylated β -cyclodextrin (Me- β -CD, with an average degree of methylation of 1.8 methyl groups per glucose unit)⁴⁵ and 2-hydroxypropyl- β -cyclodextrin (2-HPCD).⁴⁶

Fluorescence Modulation Experiments

These experiments measure the ability of the analyte to induce measurable and unique changes in the fluorescence emission spectra of a high-quantum yield fluorophore in the presence of a cyclodextrin host. The experiments were conducted by adding small amounts of a concentrated solution of the fluorophore in tetrahydrofuran to a 10 mM cyclodextrin solution in aqueous buffer, which led to a 91% aqueous/9% organic final solvent composition (volume/volume). These solvent conditions were designed to maximize hydrophobic association between the cyclodextrin hosts and small molecule guests,⁴⁷ while ensuring solubility of all organic components. The

fluorescence emission spectrum of the fluorophore under these conditions was measured and compared to the fluorescence emission spectrum of the fluorophore obtained after introduction of 20 μ L of the analyte of interest under the same conditions.

The difference between these two fluorophore emission spectra was defined as the degree of fluorescence modulation, and quantified according to Equation (1) (above). Selected results of the fluorophore modulation experiments are summarized in Table 1 and in Figures 3–5. Several aspects of these results merit further discussion.

Table 1. Fluorophore ratios of analytes **1-13** in combination with fluorophore **14**^a

Analyte	α-CD	β-CD	Me-β-CD	2-HPCD
1	1.07 $\pm$ 0.04	1.07 $\pm$ 0.02	1.15 $\pm$ 0.01	1.22 $\pm$ 0.01
2	1.02 $\pm$ 0.03	1.04 $\pm$ 0.01	1.05 $\pm$ 0.01	1.07 $\pm$ 0.01
3	1.03 $\pm$ 0.04	1.00 $\pm$ 0.01	1.07 $\pm$ 0.01	1.03 $\pm$ 0.00
4	1.04 $\pm$ 0.03	1.00 $\pm$ 0.01	1.04 $\pm$ 0.01	1.04 $\pm$ 0.01
5	1.01 $\pm$ 0.03	0.92 $\pm$ 0.04	1.02 $\pm$ 0.01	1.05 $\pm$ 0.02
6	1.10 $\pm$ 0.02	1.12 $\pm$ 0.02	0.99 $\pm$ 0.01	1.13 $\pm$ 0.06
7	1.15 $\pm$ 0.03	1.17 $\pm$ 0.02	0.98 $\pm$ 0.00	1.08 $\pm$ 0.00
8	1.08 $\pm$ 0.04	1.11 $\pm$ 0.04	0.98 $\pm$ 0.01	1.06 $\pm$ 0.01
9	0.93 $\pm$ 0.01	1.16 $\pm$ 0.02	0.95 $\pm$ 0.01	1.10 $\pm$ 0.02
10	1.18 $\pm$ 0.01	1.23 $\pm$ 0.01	1.09 $\pm$ 0.02	1.31 $\pm$ 0.01
11	0.99 $\pm$ 0.01	1.12 $\pm$ 0.01	0.94 $\pm$ 0.00	1.06 $\pm$ 0.01
12	1.14 $\pm$ 0.03	1.14 $\pm$ 0.04	0.96 $\pm$ 0.01	1.13 $\pm$ 0.03
13	1.05 $\pm$ 0.02	1.06 $\pm$ 0.02	0.99 $\pm$ 0.00	1.07 $\pm$ 0.01

^a All values were calculated according to Equation 1 and represent the average of 4 trials.

The introduction of an alcohol to a fluorophore–cyclodextrin solution leads to marked changes in the observed fluorophore emission signal. In the case of fluorophore **14**, these changes generally show up as an increase in the fluorophore emission signal in the presence of the alcohol (indicated by a ratio higher than 1.00). Aliphatic alcohols have been shown to bind to cyclodextrins under a variety of conditions,⁴⁸⁻⁵⁰ although in most instances the alcohol addition causes a decrease in the observed fluorophore emission signal. In our system, the observed fluorescence increases are a result of

multiple, analyte-specific factors:

(a) Linear alcohol analytes (**1**, **3** and **4**) assist in the formation of cyclodextrin–fluorophore–alcohol complexes, leading to higher fluorophore emission signals.^{51,52} Ethanol **4**, in particular, has been shown to form ternary complexes in 2-HPCD and to stabilize complex formation in Me- β -CD.⁵³ The addition of these alcohols also increases the cyclodextrin solubility, although the small amount of alcohol added (20 μ L in a 2.5 mL solution) means that the effect on cyclodextrin solubility is likely to be negligible.^{54,55} The two exceptions to this trend are analytes **3** and **4** in the presence of β -cyclodextrin, which cause no measurable change in the fluorophore emission signal. β -Cyclodextrin is the least water-soluble host investigated,⁵⁶ which may be a factor in establishing its limited response to these analytes.

(b) Control analytes **6** and **7** generally lead to increases in the fluorophore emission signal, with the exception of the methyl- β -cyclodextrin solutions which demonstrate no significant change. Analyte **6** is likely effecting these increases through acting as a hydrogen bond acceptor with the cyclodextrin hydroxyl groups, in line with analogous literature reports.⁵⁷ Analyte **7**, in contrast, is known to organize into hydrophobic aggregates in a highly polar, aqueous solvent system; these hydrophobic regions, in turn, are attractive regions for localizing a hydrophobic fluorophore and facilitating moderate fluorescence increases.⁵⁸

(c) Diol **5** shows limited changes in the fluorophore emission signal, with the exception of a significant fluorescence decrease observed in the presence of β -cyclodextrin. Ethylene glycol is known to hydrogen bond to cyclodextrins and lead to the self-assembly of cyclodextrin monomers into supramolecular aggregates;^{59,60} to the

extent that these cyclodextrin aggregates cause fluorophore aggregation, that would explain the observed fluorescence decreases (as many types of fluorophore aggregates are also less fluorescent than their monomeric counterparts).⁶¹

(d) Analytes **2** and **8** exhibit variable changes in the fluorophore emission signal, with no clearly observable trends. Interestingly, the structural differences between these two analytes are relatively minor, as they both contain free hydroxyl moieties and a cyclohexane ring structure. The minor differences (primary vs. secondary alcohol, presence of additional methyl group) are sufficient to lead to clear differences in the fluorophore emission ratios of compound **14** in cyclodextrin solutions with the introduction of these two analytes.

(e) Alcohol **9** is the only analyte that led to a decrease in the fluorescence of compound **14** in the presence of an α -cyclodextrin host, which correlates well with the reported ability of the alkyne moiety to bind in the sterically constrained α -cyclodextrin cavity,⁶² and highlights the fact that the fluorescence modulation results are intimately related to key structural features of the analytes. In the presence of the other cyclodextrin hosts, analyte **9** behaved similarly to most of the other investigated analytes, with an increase in fluorescence in the presence of β -cyclodextrin and 2-hydroxypropyl- β -cyclodextrin, and a moderate fluorescence decrease in the presence of methyl- β -cyclodextrin, which is likely driven by the strong association of fluorophore **14** with the methyl- β -cyclodextrin host.

(f) Alcohol **10**, the only example of an aromatic alcohol, led to increases in fluorescence for all hosts investigated, which is qualitatively similar to the results obtained with aliphatic alcohols **1** and **2**. However, the magnitude of the fluorescence

changes were markedly higher for alcohol **10** compared to the other investigated analytes, and indicates that aromatic alcohols demonstrate different intermolecular interactions that translate into easily observable differences in fluorescence modulation behaviors, in line with literature reports of aromatic guest binding in the cyclodextrin cavities.^{63,64}

(g) The behavior of fluorophore **14**-cyclodextrin complexes with the introduction of alcohol **11** resembles that obtained with introduction of alcohol **9**, with the exception of α -cyclodextrin solutions which demonstrated significant changes in the presence of alcohol **9**, but virtually no changes in the presence of alcohol **11**. These results highlight that the alkyne- α -cyclodextrin intermolecular interactions lead to unique photophysical read-out signals.

(h) Alcohol **12** demonstrates behaviors that are qualitatively similar to analyte **8**, with moderate fluorescence increases observed in the presence of α -cyclodextrin, β -cyclodextrin, and 2-hydroxypropyl- β -cyclodextrin, and slight fluorescence decreases observed in the presence of methyl- β -cyclodextrin. Interestingly, the behavior of these analytes is also similar to that of control analytes **6** and **7**, which indicates that the fluorescence modulation is driven by the hydrocarbon segments of the molecules and the resultant effects of these segments on cyclodextrin inclusion complexes, and less affected by the free hydroxyl groups in these cases.

(i) The introduction of control analyte **13** led to limited fluorescence changes in all hosts investigated, with no change observed in the methyl- β -cyclodextrin solution and limited fluorescence increases observed in other cases. These results indicate that the fluorescence changes observed in the other cases are related to specific analyte–

cyclodextrin–fluorophore intermolecular interactions rather than being solely a result of the addition of organic solvent.

The results for fluorophores **15** and **16** are markedly different from those obtained using fluorophore **14**, and reflect the fact that intermolecular interactions that lead to fluorescence modulation are exquisitely sensitive to the molecular structures of each component (see ESI for full tables with these fluorophores). For example, in the presence of methyl- β -cyclodextrin, fluorophore **15** undergoes substantial fluorescence decreases upon introduction of all analytes. These decreases are likely a result of the fact that fluorophore **15** binds strongly in the methyl- β -cyclodextrin cavity in the absence of any added alcohol; the addition of alcohols then disrupts the strong fluorophore-cyclodextrin complex and lowers the observed fluorescence. In another example, the fluorescence emission of fluorophore **16** changes very little with the introduction of analytes **1–13** to the cyclodextrin solution for all analytes in α -cyclodextrin and β -cyclodextrin, which indicates a limited interaction between the analytes and the fluorophore in these cases. Steric incompatibilities may prevent the larger fluorophore **16** from binding in the cyclodextrin cavity; as a result, the alcohol-cyclodextrin non-covalent association complexes have no effect on the photophysical properties of fluorophore **16**. These differential responses between analytes in the presence of different cyclodextrin hosts can be seen more clearly in Figures 3–5.

Figure 3. Fluorescence emission spectra of fluorophores in methyl- β -cyclodextrin in the presence of compound **1**, compound **2**, compound **3**, and compound **4**. (A) Fluorophore **14**; (B) fluorophore **15**; and (C) fluorophore **16**. (λ_{ex} compound **14** = 460 nm; compound **15** = 420 nm; compound **16** = 490 nm)

As illustrated in Figure 3, in the presence of methyl- β -cyclodextrin, compounds **1–4** effect noticeably different changes in the fluorophore emission spectra of compounds **14–16**. Compounds **3** and **4** lead to markedly higher fluorescence emissions of compound **14** compared to compounds **1** and **2** (Figure 3(A)). Fluorophore **15**, in contrast, demonstrates relative insensitivity to the presence of the aliphatic alcohols (Figure 3(B)), with minimal differences in the fluorophore emission spectra observed. Finally, fluorophore **16** demonstrates higher emission signals in the presence of compounds **3** and **1**, with relatively lower signals observed for compounds **2** and **4** (Figure 3(C)).

Figure 4. Fluorescence emission spectra of fluorophores in β -cyclodextrin in the presence of compound **1**, compound **2**, and compound **8**. (A) Fluorophore **14**; (B) fluorophore **15**; and (C) fluorophore **16**. (λ_{ex} compound **14** = 460 nm; compound **15** = 420 nm; compound **16** = 490 nm)

Figure 4 illustrates the differential responses of fluorophores **14–16** to the introduction of analytes **1**, **2** and **8** in the presence of β -cyclodextrin host. Here, noticeably different response patterns are observed for each analyte, which is striking given the structural similarities between the analytes. Furthermore, it would be difficult to distinguish analytes **1** and **2** via mass spectral analysis in a rapid time frame, due to their identical molecular weights (despite known differences in their fragmentation patterns),⁶⁵ which makes the differential fluorescence response patterns even more significant.

Figure 5. Fluorescence emission spectra of fluorophores in 2-hydroxypropyl- β -cyclodextrin in the presence of compound **6**, compound **7**, water and with no analyte. (A) Fluorophore **14**; (B) fluorophore **15**; and (C) fluorophore **16**. (λ_{ex} compound **14** = 460 nm; compound **15** = 420 nm; compound **16** = 490 nm)

Finally, Figure 5 illustrates the limited degree of fluorescence differentiation observed in the presence of analytes **6** and **7**. Relatively little changes in the fluorophore emission signals are observed upon introduction of these analytes, or with the introduction of 20 μ L of water (instead of an organic analyte). The one exception to this is the marked decrease observed in the fluorescence emission of fluorophore **16** in the presence of 20 μ L of analyte **6** (Figure 5(C)), and current efforts are focused on elucidating the reason for this aberrant behavior.

Overall, substantially different fluorescence response patterns were observed for fluorophores **14–16** in the presence of cyclodextrin hosts with the introduction of analytes **1–13**, which enables the development of selective array-based detection systems. Array-based detection has been well-established as a tool for selective analyte detection,⁶⁶ using statistical analysis such as linear discriminant analysis (LDA) and principal component analysis (PCA) to identify unique response patterns for a variety of target analytes.⁶⁷⁻⁶⁹ For the fluorescence modulation system reported herein, the integrated fluorophore emission in the presence of the target analyte ($Fl_{analyte}$, see Equation (1)) was used as the input data for the arrays. These modulation experiments generated well separated signals for **99%** of the analyte-cyclodextrin combinations investigated (Figure 6).

Figure 6. Generated array for the selective identification of alcohols in all cyclodextrins and in phosphate-buffered saline

Interestingly, the signals for cyclohexanol and 4-MCHM (analytes **2** and **8**) are grouped near each other for each cyclodextrrin host, but still generate well-separated signals in every case, highlighting their structural similarities that lead to similar response patterns in combination with cyclodextrrin hosts and high-quantum yield fluorophores. Similarly, ethanol and ethylene glycol (analytes **4** and **5**) generate signals that are close to, but well separated from, each other for each cyclodextrrin host. Finally, the signals for methanol and ethanol (analytes **3** and **4**) are found far away from each other, indicating that despite their apparent structural similarities, their interactions with cyclodextrrin hosts are in fact quite different.

Analytes **9–11** displayed signals that were relatively near each other and clearly distinct from signals generated by analytes **1–8**, highlighting that their unique structural features contribute to different intermolecular interactions with the fluorophores and cyclodextrin hosts, leading in turn to unique spectroscopic signatures. Tertiary alcohol **12**, in contrast, generated response patterns that were similar to many of the other aliphatic alcohols investigated, which indicates that the substitution around the free hydroxyl group is less important than the other structural features of the molecule such as the presence or absence of an aromatic ring or alkyne group that lead to grouping of the analytes in similar regions in the LDA-generated plots.

The practical utility of this array would be markedly enhanced by its ability to generate well-separated signals for mixtures of analytes, as nearly all instances of analyte identification in complex environments involve toxicant mixtures.⁷⁰ To that end, an array was generated using mixtures of analytes **2** and **8**, and the results are summarized in Figure 7 for fluorophore **14**. These results indicate that well-separated signals are generated for all mixtures of analytes **2** and **8** in all cyclodextrin hosts, indicating the high degree of selectivity. Moreover, the signals for the single component mixtures were relatively far away from those of the analyte mixtures, which points to the possibility of some degree of cooperativity between analytes **2** and **8** that is responsible for the observed non-linearity in array response patterns.

Figure 7. Array using mixtures of analytes **2** and **8** using all cyclodextrins and fluorophore **14**

The fundamental basis for the observed high levels of differential selectivity in these alcohol–cyclodextrin–fluorophore systems is that non-covalent interactions between the analyte and fluorophore lead to measurable, analyte-specific fluorescence changes. Control experiments using a cyclodextrin-free phosphate-buffered saline solution indicate that the presence of the cyclodextrin hosts affects the fluorescence modulation results in specific, cyclodextrin-dependent ways.

One phenomenon that is facilitated in the presence of cyclodextrin hosts is the

binding of the fluorophores in the cyclodextrin cavity, and concomitant increases in the fluorophore emission signals (Figure 8).

Figure 8. Fluorescence emission of fluorophores in various cyclodextrin hosts in the absence (A-C) and presence (D-F) of analyte **1**. (A) and (D) Fluorophore **14**; (B) and (E) Fluorophore **15**; (C) and (F) Fluorophore **16**

Fluorophore emission signals in the absence of cyclodextrin and in the presence of α -cyclodextrin are markedly lower compared to the emission signals observed in the presence of β -cyclodextrin, methyl- β -cyclodextrin, and 2-hydroxypropyl- β -cyclodextrin, which indicates that the fluorophores bind in the cavities of larger cyclodextrin hosts.⁷¹ This increase occurs both for systems without an alcohol analyte (Figure 8(A–C)) and systems that contain an alcohol additive (compound **1** used as an example; Figure 8(D–F)). This fluorophore–cyclodextrin binding is one of several intermolecular interactions that exist for each unique analyte–fluorophore–cyclodextrin combination and enables excellent differentiation between the target analytes.

The practicality of this modulation was further demonstrated through

determining the fluorescence modulation induced by analyte **8** in β -cyclodextrin dissolved in different solvent systems: ultrapure water, phosphate-buffered saline, 1 M NaCl, and crude seawater collected directly from the Narragansett Bay. The resulting ratios demonstrated solvent dependence that was translated into a highly selective array which distinguishes the analyte in different solvent environments with 100% accuracy (Table 2).

Table 2. Fluorophore ratios of analyte **8** in β -cyclodextrin^a

Analyte	Fluorophore 14	Fluorophore 15	Fluorophore 16
1 M NaCl	1.02 $\pm$ 0.01	0.97 $\pm$ 0.01	0.99 $\pm$ 0.00
Ultrapure water	1.02 $\pm$ 0.01	0.89 $\pm$ 0.00	0.96 $\pm$ 0.00
Seawater	1.06 $\pm$ 0.01	1.03 $\pm$ 0.01	0.98 $\pm$ 0.00
PBS	1.06 $\pm$ 0.01	1.03 $\pm$ 0.01	0.96 $\pm$ 0.00

^a All values were calculated according to Equation 1 and represent the average of 4 trials.

The ability of the cyclodextrin-promoted fluorescence modulation to operate in crude seawater highlights the practicality of this method in real-world environmental contamination scenarios. Practical applications of this detection method require the ability to detect alcohols at levels at or below literature-reported levels of concern.⁷² Limits of detection for all analyte–cyclodextrin combinations were calculated following literature-reported procedures, and selected results are highlighted in Table 3.

Table 3. Selected LODs for analytes **1-13** using the fluorescence modulation of fluorophore 14 in the presence of varying cyclodextrin hosts

Analyte	α-CD	β-CD	Me-β-CD	2-HPCD
1	102 μ M	<i>a</i>	<i>a</i>	109 μ M
2	18 μ M	30 μ M	402 μ M	22 μ M
3	<i>a</i>	<i>a</i>	2298 μ M	352 μ M
4	178 μ M	4 μ M	115 μ M	183 μ M
8	64 μ M	6 μ M	<i>a</i>	165 μ M
9	<i>a</i>	27 μ M	47 μ M	<i>a</i>
10	109 μ M	63 μ M	41 μ M	94 μ M
11	0.2 μ M	24 μ M	534 μ M	64 μ M
12	124 μ M	<i>a</i>	29 μ M	<i>a</i>

^a Negative values were obtained for these analyte-cyclodextrin combinations; current efforts are focused on understanding the reason for these values.

Micromolar detection limits for these analytes are a promising start towards the development of highly sensitive detection systems. One way to further improve sensitivity would be to introduce specific recognition groups to the fluorophores. Such groups would improve binding ability by promoting stronger intermolecular interactions between the analyte and fluorophore.⁷³ Efforts towards improving the sensitivity of this system are currently underway in our research group.

CONCLUSIONS

A variety of high-quantum yield fluorophores demonstrate measurable changes in their fluorescence emission spectra with introduction of small amounts of alcohols. These changes, termed ‘fluorescence modulation’, are highly specific to each analyte–fluorophore–cyclodextrin combination and can be used to develop an array with **99%** success in generating unique pattern identifiers for each analyte. Notably, small structural changes in the alcohols lead to markedly different fluorescence response patterns and unique pattern identifiers that can distinguish, for example, cyclohexanol from 1-hexanol, methanol from ethanol, and benzyl alcohol from phenol. More broadly,

different classes of alcohols such as aliphatic alcohols display signals in a region of the plot that is unique from signals obtained from propargylic, aromatic and benzylic alcohols, and point to the potential of developing class-specific signatures for different alcohol classes. Finally, the highly selective array demonstrates significant promise in detecting binary mixtures of analytes with high selectivity, a result that significantly broadens the practical applicability of this technology for the development of fluorescence-based alcohol detection systems. Efforts towards the development of such detection systems are currently underway in our laboratory, and results of these and other investigations will be reported in due course.

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Sensitive and Selective Detection of Alcohols via Fluorescence Modulation

MATERIALS AND METHODS

All analytes and cyclodextrins were obtained from Sigma-Aldrich chemical company. MCHM was obtained from Tokyo Chemical Company. Fluorophore **14**, BODIPY, was synthesized according to literature procedures. All fluorescence measurements were performed using a Shimadzu RF 5301 spectrophotometer. Both the excitation slit width and the emission slit width were 1.5 nm. All fluorescence spectra were integrated vs. wavenumber on the X-axis using OriginPro Version 9.1. All arrays were generated using SYSTAT Version 13.

DETAILS OF ANALYTES AND FLUOROPHORES

Figure S1. Structures of analytes **1-13** and fluorophores **14-16**

All analytes were prepared at a concentration of 1 mg/mL in tetrahydrofuran (THF). All fluorophores were prepared at 0.1 mg/mL in THF. The final concentrations of all analytes and fluorophores are shown in the tables below.

Table S1. Final analyte concentrations

Analyte Number	Amount Added	Final Concentration
1	20 μ L	75.3 μ M
2	20 μ L	76.8 μ M
3	20 μ L	241 μ M
4	20 μ L	167 μ M
5	20 μ L	124 μ M
6	20 μ L	104 μ M
7	20 μ L	89.3 μ M
8	20 μ L	59.9 μ M
9	20 μ L	137 μ M
10	20 μ L	81.7 μ M
11	20 μ L	71.1 μ M
12	20 μ L	104 μ M
13	20 μ L	107 μ M

Table S2. Final fluorophore concentrations

Fluorophore Number	Amount Added	Final Concentration
14	100 μ L	9.5 μ M
15	100 μ L	11 μ M
16	100 μ L	8.1 μ M

SYNTHESIS OF FLUOROPHORE **14**

The synthesis of BODIPY **14** was performed according to literature procedures.

REFERENCE: Shepherd, J. L.; Kell, A.; Chung, E.; Sinclar, C. W.; Workentin, M. S.; Bizzotto, D. *J. Am. Chem. Soc.* **2004**, *126*, 8329-8335.

Reaction 1:

Figure S2. Synthetic pathway to structure **S5**

Procedure: 2.0 grams of 11-bromoundecanoic acid **S1** (7.54 mmol, 1.0 eq.) was combined with 2 drops of *N,N*-dimethylformamide in 40 mL of dichloromethane. 1.0 gram of oxalyl chloride **S2** (7.88 mmol, 1.05 eq.) was dissolved in 5.0 mL of dichloromethane and added dropwise. The reaction mixture was stirred for one hour, then the crude mixture was concentrated on the rotary evaporator and dried on a vacuum overnight to remove any unreacted oxalyl chloride. The resulting acid chloride **S3** was dissolved in 50 mL of dichloromethane. 0.772 mL of 2,4-dimethylpyrrole **S4** (7.50 mmol, 0.99 eq.) was dissolved in 5.0 mL of dichloromethane and added to the reaction mixture. The resulting reaction mixture was heated to reflux for 3 hours under a nitrogen atmosphere, during which time the mixture became a dark red color. After three hours, the reaction mixture was cooled to room temperature and solvent was removed on the rotary evaporator until approximately 5.0 mL of the dichloromethane solution remained. 200 mL of *n*-hexanes were added to the flask, and the mixture was cooled overnight in

the freezer at -20 °C. The hexanes were decanted from the insoluble oil and precipitate. The resulting crude product was dissolved in 75 mL of toluene and heated to 80 °C. 1.0 mL of triethylamine (7.17 mmol, 0.95 eq.) was added and the solution immediately turned light yellow. 1.0 mL of boron trifluoride etherate (8.10 mmol, 1.07 eq.) was then added and the reaction mixture was stirred at 80 °C for 30 minutes, during which time the color of the mixture darkened and became fluorescent. The reaction mixture was cooled to room temperature, and the product was extracted 3 times with brine (50 mL each time). The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography (1:1 dichloromethane: hexanes) to yield the desired product in 28% yield (comparable to the literature-reported 24% yield).

Reaction 2:

Figure S3. Synthetic pathway to structure **S7**

Procedure: Compound **S5** (0.968 g, 2.07 mmol, 1.0 eq.) and compound **S6** (0.27 grams, 2.36 mmol, 1.14 eq.) were dissolved in 50 mL of acetone. The reaction mixture was heated to reflux for two hours. After two hours, the reaction mixture was cooled to room temperature, acetone was removed, and the crude solid was re-dissolved in dichloromethane and washed with water. The organic extract was dried over sodium sulfate, filtered and concentrated, to yield compound **S7** in 97% yield (0.932 grams).

Reaction 3:

Figure S4. Synthetic pathway to compound **14**

Procedure: Compound **S7** (0.932 grams, 2.01 mmol, 1.0 eq.) was dissolved in 150 mL of anhydrous ethanol that was purged with nitrogen. Potassium carbonate was added, and the reaction mixture was warmed to 30 °C. The reaction mixture was stirred under nitrogen for 4 hours at 30 °C. The contents of the flask were poured over 40 mL of aqueous saturated ammonium chloride, at which point the solution turned bright orange. The product was extracted with dichloromethane and washed several times with water. The organic layer was dried over sodium sulfate, filtered, and concentrated. The product was purified via flash chromatography (1:1 dichloromethane: hexanes) to yield compound **14** in 76% yield (674 mg).

EXPERIMENTAL DETAILS FOR FLUORESCENCE MODULATION EXPERIMENTS

In a quartz cuvette, 2.5 mL of a 10 mM cyclodextrin solution in phosphate-buffered saline (PBS) and 100 μ L of a fluorophore **14** solution (0.1 mg/mL in THF) were combined. The solution was excited at 460 nm, which is the excitation wavelength to directly excite **14**. 20 μ L of a 1 mg/mL analyte solution in THF was added to the cuvette, and fluorescence measurements were repeated at 460 nm. The experiments were repeated for fluorophore **15** and **16** at excitation wavelengths of 420 nm and 490 nm, respectively. The fluorescence spectra were integrated vs. wavenumber on the X-axis, and fluorescence modulation was determined by quantifying a fluorophore ratio according to Equation 1:

$$\text{Fluorophore Ratio} = (F_{\text{alcohol}})/F_{\text{blank}} \quad (\text{Equation 1})$$

where F_{alcohol} is the fluorescence emission of the fluorophore in the presence of the alcohol, and F_{blank} is the fluorescence emission of the fluorophore in the absence of the alcohol. Each experiment was repeated four times. The reported values are the average of the results.

EXPERIMENTAL DETAILS FOR LIMIT OF DETECTION EXPERIMENTS

The limit of detection (LOD) is defined as the lowest concentration of analyte at which a signal can be detected. The limit of quantification is defined at the lowest concentration of analyte that can be accurately quantified. These experiments were conducted following literature-reported procedures:

REFERENCE: Saute, B.; Premasiri, R.; Ziegler, L.; Narayanan, R. "Gold Nanorods as Surface Enhanced Raman Spectroscopy Substrates for Sensitive and Selective Detection of Ultra-Low Levels of Dithiocarbamate Pesticides." *Analyst* **2012**, *137*, 5082-5087.

To determine the limit of detection (LOD) and limit of quantification (LOQ), each fluorophore-analyte-cyclodextrin combination was examined in the following manner:

1. 2.5 mL of 10 mM cyclodextrin in phosphate-buffered saline (PBS) was measured into a cuvette and 100 μ L of a fluorophore solution (0.1 mg/mL) in THF was added. The solution was excited at the fluorophore's excitation wavelength and the fluorescence emission spectrum was recorded. Six repeat measurements were made for the fluorescence emission spectra.
2. 20 μ L of the analyte was added to the cuvette and the solution was again excited at the fluorophore's excitation wavelength. Six repeat measurements were taken.
3. Step 2 was repeated for 40 μ L of analyte, 60 μ L of analyte, 80 μ L of analyte, and 100 μ L of analyte. In each case, the solution was excited at the fluorophore's excitation wavelength and the fluorescence emission spectrum was recorded four times.
4. All fluorescence emission spectra were integrated vs. wavenumber, and calibration curves were generated with the analyte concentration on the X-axis (in μ M) and the integrated fluorophore emission on the Y-axis. The curve was then fitted to a straight line and an equation for the line was determined.

5. For each case, the fluorophore with cyclodextrin (before any analyte was added) was also excited at its excitation wavelength, and the fluorescence emission spectrum was recorded (as per step 1). These measurements are referred to as the “blank.”

6. The limit of the blank is defined according to the following equation:

$$LoBLOD = m_{blank} + 3(SD_{blank})$$

Where m is the mean of the blank integrations and SD is the standard deviation.

7. The limit of the blank was then entered into the equation determined in step 4 (for the y value), and the corresponding X value was determined. This value provided the LOD in μM .

8. The limit of quantification (LOQ) was calculated in a similar way to the limit of detection. First, the limit of the blank for quantification was determined according to the following equation:

$$LoBLOQ = m_{blank} + 10(SD_{blank})$$

This value was entered into the equation determined in step 4 (for the y value), and the corresponding X value was determined to be the limit of quantification in μM .

EXPERIMENTAL DETAILS FOR ARRAY GENERATION EXPERIMENTS

Array analysis was performed using SYSTAT 13 statistical computing software with the following settings: (a) Classical Discriminant Analysis; (b) Grouping Variable: Analytes; (c) Predictors: Bodipy, Rhodamine 6G, Coumarin 6; (d) Long-Range Statistics: Mahal

Arrays were generated for: (a) all analyte-fluorophore-cyclodextrin combinations in one array; and (b) individual cyclodextrin hosts in separate arrays. The results are summarized in the tables.

SUMMARY TABLES FOR FLUORESCENCE MODULATION EXPERIMENTS

Table S3. Fluorophore ratios of analytes **1-13** in combination with fluorophore **15^a**

Analyte	α -CD	β -CD	Me- β -CD	2-HPCD
1	1.09 $\pm$ 0.01	1.01 $\pm$ 0.01	0.60 $\pm$ 0.14	0.99 $\pm$ 0.02
2	1.08 $\pm$ 0.01	1.04 $\pm$ 0.01	0.68 $\pm$ 0.14	0.17 $\pm$ 0.04
3	1.09 $\pm$ 0.00	0.98 $\pm$ 0.01	0.71 $\pm$ 0.14	0.21 $\pm$ 0.06
4	1.13 $\pm$ 0.01	1.08 $\pm$ 0.03	0.75 $\pm$ 0.06	1.04 $\pm$ 0.07
5	1.01 $\pm$ 0.01	1.00 $\pm$ 0.02	0.83 $\pm$ 0.08	0.97 $\pm$ 0.02
6	1.05 $\pm$ 0.01	0.95 $\pm$ 0.01	0.92 $\pm$ 0.00	0.88 $\pm$ 0.01
7	0.92 $\pm$ 0.02	0.87 $\pm$ 0.02	0.90 $\pm$ 0.01	0.86 $\pm$ 0.01
8	1.02 $\pm$ 0.02	0.92 $\pm$ 0.01	0.81 $\pm$ 0.01	0.85 $\pm$ 0.01
9	0.95 $\pm$ 0.00	0.26 $\pm$ 0.07	0.97 $\pm$ 0.00	0.65 $\pm$ 0.05
10	1.22 $\pm$ 0.01	0.37 $\pm$ 0.12	0.94 $\pm$ 0.00	0.73 $\pm$ 0.06
11	1.07 $\pm$ 0.01	0.53 $\pm$ 0.20	0.96 $\pm$ 0.00	0.81 $\pm$ 0.04
12	1.03 $\pm$ 0.01	0.49 $\pm$ 0.21	0.97 $\pm$ 0.00	0.75 $\pm$ 0.04
13	1.07 $\pm$ 0.01	0.88 $\pm$ 0.01	0.98 $\pm$ 0.00	0.97 $\pm$ 0.01

^a All values were calculated according to Equation 1 and represent the average of 4 trials.

Table S4. Fluorophore ratios of analytes **1-13** in combination with fluorophore **16**^a

Analyte	α -CD	β -CD	Me- β -CD	2-HPCD
1	1.00 $\pm$ 0.01	1.03 $\pm$ 0.01	1.00 $\pm$ 0.00	1.01 $\pm$ 0.01
2	1.01 $\pm$ 0.01	0.98 $\pm$ 0.02	1.17 $\pm$ 0.02	1.02 $\pm$ 0.01
3	1.01 $\pm$ 0.01	1.03 $\pm$ 0.01	1.39 $\pm$ 0.03	1.09 $\pm$ 0.00
4	1.03 $\pm$ 0.02	1.00 $\pm$ 0.02	1.17 $\pm$ 0.01	0.99 $\pm$ 0.01
5	1.03 $\pm$ 0.02	1.00 $\pm$ 0.01	1.08 $\pm$ 0.01	1.07 $\pm$ 0.01
6	1.01 $\pm$ 0.01	1.05 $\pm$ 0.02	1.14 $\pm$ 0.03	1.03 $\pm$ 0.01
7	0.97 $\pm$ 0.01	0.96 $\pm$ 0.02	1.00 $\pm$ 0.01	0.98 $\pm$ 0.01
8	0.97 $\pm$ 0.01	0.99 $\pm$ 0.01	0.99 $\pm$ 0.02	0.99 $\pm$ 0.01
9	1.01 $\pm$ 0.00	1.00 $\pm$ 0.00	0.99 $\pm$ 0.00	1.01 $\pm$ 0.00
10	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	0.99 $\pm$ 0.00	1.00 $\pm$ 0.00
11	0.97 $\pm$ 0.00	0.98 $\pm$ 0.00	0.99 $\pm$ 0.00	1.00 $\pm$ 0.00
12	0.99 $\pm$ 0.00	0.98 $\pm$ 0.00	0.98 $\pm$ 0.00	0.99 $\pm$ 0.01
13	0.98 $\pm$ 0.00	0.98 $\pm$ 0.00	0.98 $\pm$ 0.00	0.99 $\pm$ 0.01

^a All values were calculated according to Equation 1 and represent the average of 4 trials.

**SUMMARY TABLE FOR DIFFERENT SOLVENT SYSTEMS USING
ANALYTE 8 IN β -CYCLODEXTRIN**

Table S5. Summary table for different solvent systems using analyte **8** in β -cyclodextrin

	Fluorophore 14	Fluorophore 15	Fluorophore 16
1 M NaCl	1.02 ± 0.01	0.97 ± 0.01	0.99 ± 0.00
ultrapure water	1.02 ± 0.01	0.89 ± 0.00	0.96 ± 0.00
seawater	1.06 ± 0.01	1.03 ± 0.01	0.98 ± 0.00
PBS	1.06 ± 0.01	1.03 ± 0.01	0.96 ± 0.00

SUMMARY TABLES FOR LIMIT OF DETECTION EXPERIMENTS

Table S6. Summary table for limit of detection experiments

Analyte	Fluorophore	CD	LOD (μM)	LOQ (μM)	Equation	R ²
1	14	α -CD	102.2	204.6	$y = 52.897x + 35048$	0.9972
1	14	β -CD	<i>a</i>	43.8	$y = 55.104x + 49523$	0.9846
1	14	Me- β -CD	<i>a</i>	77.0	$y = 77.26x + 82316$	0.9999
1	14	2-HPCD	109.2	200.4	$y = 76.811x + 43207$	0.9984
1	14	PBS	8.0	46.7	$y = 87.498x + 43572$	0.9976
2	14	α -CD	18.0	192.3	$y = 42.312x + 46434$	0.9784
2	14	β -CD	30.1	71.2	$y = 73.443x + 49933$	0.9923
2	14	Me- β -CD	401.6	427.9	$y = 13.153x + 85888$	0.9639
2	14	2-HPCD	21.5	82.1	$y = 62.827x + 52136$	0.9994
2	14	PBS	12.2	15.4	$y = 103.59x + 42017$	0.9998
3	14	α -CD	<i>a</i>	14256.0	$y = 2.0296x + 107479$	0.9079
3	14	β -CD	<i>a</i>	221.0	$y = 26.049x + 63193$	0.9988
3	14	Me- β -CD	2298.0	2383.0	<i>b</i>	<i>b</i>
3	14	2-HPCD	352.0	677.0	$y = 25.508x + 61113$	0.9963
3	14	PBS	<i>a</i>	185.0	$y = 38.138x + 49325$	0.9888
3	15	α -CD	151.0	264.0	$y = 22.966x + 66914$	0.9951
3	15	β -CD	<i>a</i>	<i>a</i>	$y = -24.062x + 135707$	0.9448
3	15	Me- β -CD	<i>a</i>	<i>a</i>	$y = -463.38x + 1\text{E}6$	0.9999
3	15	2-HPCD	223.0	170.0	$y = -251.97x + 407239$	0.9825
3	15	PBS	201.0	291.0	$y = 23.444x + 62467$	0.9970
3	16	α -CD	<i>a</i>	<i>a</i>	$y = -67.888x + 1\text{E}6$	0.9943
3	16	β -CD	<i>a</i>	<i>a</i>	$y = -67.49x + 1\text{E}6$	0.9760
3	16	Me- β -CD	<i>a</i>	<i>a</i>	$y = -44.365x + 1\text{E}6$	0.9879
3	16	2-HPCD	<i>a</i>	<i>a</i>	$y = -25.44x + 1\text{E}6$	0.8746
3	16	PBS	<i>a</i>	<i>a</i>	$y = -31.313x + 770331$	0.7262
4	14	α -CD	178.0	1204.0	$y = 23.887X + 55946$	0.9779
4	14	β -CD	4.0	192.0	$y = 40.425x + 55708$	0.9979
4	14	Me- β -CD	115.0	19.0	$y = -13.83x + 112839$	0.8195
4	14	2-HPCD	183.0	318.0	$y = 44.927x + 52002$	0.9842
4	14	PBS	51.0	278.0	$y = 58.209x + 41162$	0.9992
4	15	α -CD	80.0	123.0	$y = 35.54x + 57762$	0.9841
4	15	β -CD	<i>a</i>	<i>a</i>	$y = -25.176x + 121103$	0.8862
4	15	Me- β -CD	<i>a</i>	<i>a</i>	$y = -743.59x + 835393$	0.9735
4	15	2-HPCD	128.0	26.0	$y = -262.47x + 433210$	0.9981
4	15	PBS	111.0	121.0	$y = 46.214x + 63696$	0.9686
4	16	α -CD	18.0	<i>a</i>	$y = -35.189x + 391701$	0.9991
4	16	β -CD	<i>a</i>	<i>a</i>	$y = -18.992x + 400576$	0.9957
4	16	Me- β -CD	<i>a</i>	<i>a</i>	$y = -9.7123x + 388109$	0.7445
4	16	2-HPCD	1119104.0	1124473.0	$y = -39.323x + 1\text{E}6$	0.9875
4	16	PBS	1113038.0	1148325.0	$y = -66.674x + 1\text{E}6$	0.9982

Analyte	Fluorophore	CD	LOD (μM)	LOQ (μM)	Equation	R ²
8	14	α -CD	63.6	189.2	$y = 18.494x + 25641$	0.9248
8	14	β -CD	6.3	27.7	$y = 76.95x + 39029$	0.9957
8	14	Me- β -CD	<i>a</i>	<i>a</i>	$y = -26.172x + 88805$	0.9205
8	14	2-HPCD	164.6	353.7	$y = 26.306x + 46582$	0.9414
8	14	PBS	12.5	73.4	$y = 68.756x + 39383$	0.9979
9	14	α -CD	<i>a</i>	1158.8	$y = 10.276x + 75615$	0.8908
9	14	β -CD	27.1	98.3	$y = 32.857x + 74297$	0.9988
9	14	Me- β -CD	46.7	368.7	$y = 21.225x + 74017$	0.9990
9	14	2-HPCD	<i>a</i>	257.8	$y = 38.874x + 71234$	0.9982
9	14	PBS	<i>a</i>	225.4	$y = 26.092x + 69625$	0.9893
10	14	α -CD	108.9	302.5	$y = 51.534x + 63540$	0.9955
10	14	β -CD	63.3	172.1	$y = 87.661x + 46279$	0.9952
10	14	Me- β -CD	40.8	81.9	$y = 44.784x + 58935$	0.9986
10	14	2-HPCD	94.4	233.4	$y = 112.43x + 48688$	0.9911
10	14	PBS	109.9	263.8	$y = 40.689x + 62051$	0.9945
11	14	α -CD	0.2	84.7	$y = 75.754x + 51142$	1.0000
11	14	β -CD	23.8	89.2	$y = 99.09x + 36149$	0.9998
11	14	Me- β -CD	534.1	573.7	$y = 14.777x + 51151$	0.9743
11	14	2-HPCD	63.7	150.2	$y = 106.59x + 44981$	0.9984
11	14	PBS	<i>a</i>	61.0	$y = 98.664x + 42088$	0.9975
12	14	α -CD	123.5	231.5	$y = 54.457x + 70545$	0.964
12	14	β -CD	<i>a</i>	159.0	$y = 75.572x + 68689$	0.9924
12	14	Me- β -CD	29.1	115.1	$y = 82.904x + 65333$	1
12	14	2-HPCD	<i>a</i>	159.7	$y = 45.603x + 70779$	0.9985
12	14	PBS	<i>a</i>	294.5	$y = 25.25x + 65219$	0.9835
<i>a</i> = nonsensical values obtained						
<i>b</i> = non-linear fit						

SUMMARY TABLES FOR ARRAY DATA

Table S7. Results of array generation for analytes **1-13** with all cyclodextrin hosts with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	2HPCD1-hexanol	2HPCDMCHM	2HPCDTHF	2HPCDbenzylalcohol	2HPCDcyclohexanol	2HPCDethanol	2HPCDethyleneglycol
2HPCD1-hexanol	4	0	0	0	0	0	0
2HPCDMCHM	0	4	0	0	0	0	0
2HPCDTHF	0	0	4	0	0	0	0
2HPCDbenzylalcohol	0	0	0	4	0	0	0
2HPCDcyclohexanol	0	0	0	0	4	0	0
2HPCDethanol	0	0	0	0	0	4	0
2HPCDethyleneglycol	0	0	0	0	0	0	4
2HPCDmethanol	0	0	0	0	0	0	0
2HPCDphenol	0	0	0	0	0	0	0
2HPCDpropargylalcohol	0	0	0	0	0	0	0
2HPCDtertiarybutanol	0	0	0	0	0	0	0
ACD1-hexanol	0	0	0	0	0	0	0
ACDMCHM	0	0	0	0	0	0	0
ACDTHF	0	0	0	0	0	0	0
ACDbenzylalcohol	0	0	0	0	0	0	0
ACDcyclohexanol	0	0	0	0	0	0	0
ACDethanol	0	0	0	0	0	0	0
ACDethyleneglycol	0	0	0	0	0	0	0
ACDmethanol	0	0	0	0	0	0	0
ACDphenol	0	0	0	0	0	0	0

ACDpropargylalcohol	0	0	0	0	0	0	0
ACDtertiarybutanol	0	0	0	0	0	0	0
BCD1-hexanol	0	0	0	0	0	0	0
BCDMCHM	0	0	0	0	0	0	0
BCDTHF	0	0	0	0	0	0	0
BCDbenzylalcohol	0	0	0	0	0	0	0
BCDcyclohexanol	0	0	0	0	0	0	0
BCDethanol	0	0	0	0	0	0	0
BCDethyleneglycol	0	0	0	0	0	0	0
BCDmethanol	0	0	0	0	0	0	0
BCDphenol	0	0	0	0	0	0	0
BCDpropargylalcohol	0	0	0	0	0	0	0
BCDtertiarybutanol	0	0	0	0	0	0	0
MeBCD1-hexanol	0	0	0	0	0	0	0
MeBCDMCHM	0	0	0	0	0	0	0
MeBCDTHF	0	0	0	0	0	0	0
MeBCDbenzylalcohol	0	0	0	0	0	0	0
MeBCDcyclohexanol	0	0	0	0	0	0	0
MeBCDethanol	0	0	0	0	0	0	0
MeBCDethyleneglycol	0	0	0	0	0	0	0
MeBCDmethanol	0	0	0	0	0	0	0
MeBCDphenol	0	0	0	0	0	0	0
MeBCDpropargylalcohol	0	0	0	0	0	0	0
MeBCDtertiarybutanol	0	0	0	0	0	0	0

PBS1-hexanol	0	0	0	0	0	0	0
PBSMCHM	0	0	0	0	0	0	0
PBSTHF	0	0	0	0	0	0	0
PBSbenzylalcohol	0	0	0	0	0	0	0
PBScyclohexanol	0	0	0	0	0	0	0
PBSethanol	0	0	0	0	0	0	0
PBSethyleneglycol	0	0	0	0	0	0	0
PBSmethanol	0	0	0	0	0	0	0
PBSphenol	0	0	0	0	0	0	0
PBSpropargylalcohol	0	0	0	0	0	0	0
PBStertiarybutanol	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	2HPCDmethanol	2HPCDphenol	2HPCDpropargylalcohol	2HPCDtertiarybutanol	ACD1-hexanol	ACDMCHM	ACDTHF	ACDbenzylalcohol
2HPCD1-hexanol	0	0	0	0	0	0	0	0
2HPCDMCHM	0	0	0	0	0	0	0	0
2HPCDTHF	0	0	0	0	0	0	0	0
2HPCDbenzylalcohol	0	0	0	0	0	0	0	0
2HPCDcyclohexanol	0	0	0	0	0	0	0	0
2HPCDethanol	0	0	0	0	0	0	0	0
2HPCDethyleneglycol	0	0	0	0	0	0	0	0
2HPCDmethanol	4	0	0	0	0	0	0	0
2HPCDphenol	0	4	0	0	0	0	0	0
2HPCDpropargylalcohol	0	0	4	0	0	0	0	0
2HPCDtertiarybutanol	0	0	0	4	0	0	0	0
ACD1-hexanol	0	0	0	0	4	0	0	0
ACDMCHM	0	0	0	0	0	4	0	0
ACDTHF	0	0	0	0	0	0	4	0
ACDbenzylalcohol	0	0	0	0	0	0	0	4
ACDcyclohexanol	0	0	0	0	0	0	0	0
ACDethanol	0	0	0	0	0	0	0	0
ACDethyleneglycol	0	0	0	0	0	0	0	0
ACDmethanol	0	0	0	0	0	0	0	0
ACDphenol	0	0	0	0	0	0	0	0
ACDpropargylalcohol	0	0	0	0	0	0	0	0

ACDtertiarybutanol	0	0	0	0	0	0	0	0
BCD1-hexanol	0	0	0	0	0	0	0	0
BCDMCHM	0	0	0	0	0	0	0	0
BCDTHF	0	0	0	0	0	0	0	0
BCDbenzylalcohol	0	0	0	0	0	0	0	0
BCDcyclohexanol	0	0	0	0	0	0	0	0
BCDethanol	0	0	0	0	0	0	0	0
BCDethyleneglycol	0	0	0	0	0	0	0	0
BCDmethanol	0	0	0	0	0	0	0	0
BCDphenol	0	0	0	0	0	0	0	0
BCDpropargylalcohol	0	0	0	0	0	0	0	0
BCDtertiarybutanol	0	0	0	0	0	0	0	0
MeBCD1-hexanol	0	0	0	0	0	0	0	0
MeBCDMCHM	0	0	0	0	0	0	0	0
MeBCDTHF	0	0	0	0	0	0	0	0
MeBCDbenzylalcohol	0	0	0	0	0	0	0	0
MeBCDcyclohexanol	0	0	0	0	0	0	0	0
MeBCDethanol	0	0	0	0	0	0	0	0
MeBCDethyleneglycol	0	0	0	0	0	0	0	0
MeBCDmethanol	0	0	0	0	0	0	0	0
MeBCDphenol	0	0	0	0	0	0	0	0
MeBCDpropargylalcohol	0	0	0	0	0	0	0	0
MeBCDtertiarybutanol	0	0	0	0	0	0	0	0

PBS1-hexanol	0	0	0	0	0	0	0	0
PBSMCHM	0	0	0	0	0	0	0	0
PBSTHF	0	0	0	0	0	0	0	0
PBSbenzylalcohol	0	0	0	0	0	0	0	0
PBScyclohexanol	0	0	0	0	0	0	0	0
PBSethanol	0	0	0	0	0	0	0	0
PBSethyleneglycol	0	0	0	0	0	0	0	0
PBSmethanol	0	0	0	0	0	0	0	0
PBSphenol	0	0	0	0	0	0	0	0
PBSpropargylalcohol	0	0	0	0	0	0	0	0
PBStertiarybutanol	0	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	ACDcyclohexanol	ACDethanol	ACDethyleneglycol	ACDmethanol	ACDphenol	ACDpropargylalcohol	ACDtertiarybutanol
2HPCD1-hexanol	0	0	0	0	0	0	0
2HPCDMCHM	0	0	0	0	0	0	0
2HPCDTHF	0	0	0	0	0	0	0
2HPCDbenzylalcohol	0	0	0	0	0	0	0
2HPCDcyclohexanol	0	0	0	0	0	0	0
2HPCDethanol	0	0	0	0	0	0	0
2HPCDethyleneglycol	0	0	0	0	0	0	0
2HPCDmethanol	0	0	0	0	0	0	0
2HPCDphenol	0	0	0	0	0	0	0
2HPCDpropargylalcohol	0	0	0	0	0	0	0
2HPCDtertiarybutanol	0	0	0	0	0	0	0
ACD1-hexanol	0	0	0	0	0	0	0
ACDMCHM	0	0	0	0	0	0	0
ACDTHF	0	0	0	0	0	0	0
ACDbenzylalcohol	0	0	0	0	0	0	0
ACDcyclohexanol	4	0	0	0	0	0	0
ACDethanol	0	4	0	0	0	0	0
ACDethyleneglycol	0	0	4	0	0	0	0
ACDmethanol	0	0	0	4	0	0	0
ACDphenol	0	0	0	0	4	0	0

ACDpropargylalcohol	0	0	0	0	0	4	0
ACDtertiarybutanol	0	0	0	0	0	0	4
BCD1-hexanol	0	0	0	0	0	0	0
BCDMCHM	0	0	0	0	0	0	0
BCDTHF	0	0	0	0	0	0	0
BCDbenzylalcohol	0	0	0	0	0	0	0
BCDcyclohexanol	0	0	0	0	0	0	0
BCDethanol	0	0	0	0	0	0	0
BCDethyleneglycol	0	0	0	0	0	0	0
BCDmethanol	0	0	0	0	0	0	0
BCDphenol	0	0	0	0	0	0	0
BCDpropargylalcohol	0	0	0	0	0	0	0
BCDtertiarybutanol	0	0	0	0	0	0	0
MeBCD1-hexanol	0	0	0	0	0	0	0
MeBCDMCHM	0	0	0	0	0	0	0
MeBCDTHF	0	0	0	0	0	0	0
MeBCDbenzylalcohol	0	0	0	0	0	0	0
MeBCDcyclohexanol	0	0	0	0	0	0	0
MeBCDethanol	0	0	0	0	0	0	0
MeBCDethyleneglycol	0	0	0	0	0	0	0
MeBCDmethanol	0	0	0	0	0	0	0
MeBCDphenol	0	0	0	0	0	0	0

MeBCDpropargylalcohol	0	0	0	0	0	0	0
MeBCDtertiarybutanol	0	0	0	0	0	0	0
PBS1-hexanol	0	0	0	0	0	0	0
PBSMCHM	0	0	0	0	0	0	0
PBSTHF	0	0	0	0	0	0	0
PBSbenzylalcohol	0	0	0	0	0	0	0
PBScyclohexanol	0	0	0	0	0	0	0
PBSethanol	0	0	0	0	0	0	0
PBSethyleneglycol	0	0	0	0	0	0	0
PBSmethanol	0	0	0	0	0	0	0
PBSphenol	0	0	0	0	0	0	0
PBSpropargylalcohol	0	0	0	0	0	0	0
PBStertiarybutanol	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	BCD1-hexanol	BCDMCHM	BCDTHF	BCDbenzylalcohol	BCDcyclohexanol	BCDethanol	BCDethyleneglycol	BCDmethanol
2HPCD1-hexanol	0	0	0	0	0	0	0	0
2HPCDMCHM	0	0	0	0	0	0	0	0
2HPCDTHF	0	0	0	0	0	0	0	0
2HPCDbenzylalcohol	0	0	0	0	0	0	0	0
2HPCDcyclohexanol	0	0	0	0	0	0	0	0
2HPCDethanol	0	0	0	0	0	0	0	0
2HPCDethyleneglycol	0	0	0	0	0	0	0	0
2HPCDmethanol	0	0	0	0	0	0	0	0
2HPCDphenol	0	0	0	0	0	0	0	0
2HPCDpropargylalcohol	0	0	0	0	0	0	0	0
2HPCDtertiarybutanol	0	0	0	0	0	0	0	0
ACD1-hexanol	0	0	0	0	0	0	0	0
ACDMCHM	0	0	0	0	0	0	0	0
ACDTHF	0	0	0	0	0	0	0	0
ACDbenzylalcohol	0	0	0	0	0	0	0	0
ACDcyclohexanol	0	0	0	0	0	0	0	0
ACDethanol	0	0	0	0	0	0	0	0
ACDethyleneglycol	0	0	0	0	0	0	0	0

ACDmethanol	0	0	0	0	0	0	0	0
ACDphenol	0	0	0	0	0	0	0	0
ACDpropargylalcohol	0	0	0	0	0	0	0	0
ACDtertiarybutanol	0	0	0	0	0	0	0	0
BCD1-hexanol	4	0	0	0	0	0	0	0
BCDMCHM	0	3	0	0	0	0	0	0
BCDTHF	0	0	4	0	0	0	0	0
BCDbenzylalcohol	0	0	0	4	0	0	0	0
BCDcyclohexanol	0	0	0	0	4	0	0	0
BCDethanol	0	0	0	0	0	4	0	0
BCDethyleneglycol	0	0	0	0	0	0	4	0
BCDmethanol	0	0	0	0	0	0	0	4
BCDphenol	0	0	0	0	0	0	0	0
BCDpropargylalcohol	0	0	0	0	0	0	0	0
BCDtertiarybutanol	0	0	0	0	0	0	0	0
MeBCD1-hexanol	0	0	0	0	0	0	0	0
MeBCDMCHM	0	0	0	0	0	0	0	0
MeBCDTHF	0	0	0	0	0	0	0	0
MeBCDbenzylalcohol	0	0	0	0	0	0	0	0
MeBCDcyclohexanol	0	0	0	0	0	0	0	0
MeBCDethanol	0	0	0	0	0	0	0	0
MeBCDethyleneglycol	0	0	0	0	0	0	0	0

MeBCDmethanol	0	0	0	0	0	0	0	0
MeBCDphenol	0	0	0	0	0	0	0	0
MeBCDpropargylalcohol	0	0	0	0	0	0	0	0
MeBCDtertiarybutanol	0	0	0	0	0	0	0	0
PBS1-hexanol	0	0	0	0	0	0	0	0
PBSMCHM	0	1	0	0	0	0	0	0
PBSTHF	0	0	0	0	0	0	0	0
PBSbenzylalcohol	0	0	0	0	0	0	0	0
PBScyclohexanol	0	0	0	0	0	0	0	0
PBSethanol	0	0	0	0	0	0	0	0
PBSethyleneglycol	0	0	0	0	0	0	0	0
PBSmethanol	0	0	0	0	0	0	0	0
PBSphenol	0	0	0	0	0	0	0	0
PBSpropargylalcohol	0	0	0	0	0	0	0	0
PBStertiarybutanol	0	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	BCDphenol	BCDpropargylalcohol	BCDtertiarybutanol	MeBCD1-hexanol	MeBCDMCHM	MeBCDTHF	MeBCDbenzylalcohol
2HPCD1-hexanol	0	0	0	0	0	0	0
2HPCDMCHM	0	0	0	0	0	0	0
2HPCDTHF	0	0	0	0	0	0	0
2HPCDbenzylalcohol	0	0	0	0	0	0	0
2HPCDcyclohexanol	0	0	0	0	0	0	0
2HPCDethanol	0	0	0	0	0	0	0
2HPCDethyleneglycol	0	0	0	0	0	0	0
2HPCDmethanol	0	0	0	0	0	0	0
2HPCDphenol	0	0	0	0	0	0	0
2HPCDpropargylalcohol	0	0	0	0	0	0	0
2HPCDtertiarybutanol	0	0	0	0	0	0	0
ACD1-hexanol	0	0	0	0	0	0	0
ACDMCHM	0	0	0	0	0	0	0
ACDTHF	0	0	0	0	0	0	0
ACDbenzylalcohol	0	0	0	0	0	0	0
ACDcyclohexanol	0	0	0	0	0	0	0
ACDethanol	0	0	0	0	0	0	0
ACDethyleneglycol	0	0	0	0	0	0	0
ACDmethanol	0	0	0	0	0	0	0

ACDphenol	0	0	0	0	0	0	0
ACDpropargylalcohol	0	0	0	0	0	0	0
ACDtertiarybutanol	0	0	0	0	0	0	0
BCD1-hexanol	0	0	0	0	0	0	0
BCDMCHM	0	0	0	0	0	0	0
BCDTHF	0	0	0	0	0	0	0
BCDbenzylalcohol	0	0	0	0	0	0	0
BCDcyclohexanol	0	0	0	0	0	0	0
BCDethanol	0	0	0	0	0	0	0
BCDethyleneglycol	0	0	0	0	0	0	0
BCDmethanol	0	0	0	0	0	0	0
BCDphenol	4	0	0	0	0	0	0
BCDpropargylalcohol	0	4	0	0	0	0	0
BCDtertiarybutanol	0	0	4	0	0	0	0
MeBCD1-hexanol	0	0	0	4	0	0	0
MeBCDMCHM	0	0	0	0	4	0	0
MeBCDTHF	0	0	0	0	0	4	0
MeBCDbenzylalcohol	0	0	0	0	0	0	4
MeBCDcyclohexanol	0	0	0	0	0	0	0
MeBCDethanol	0	0	0	0	0	0	0
MeBCDethyleneglycol	0	0	0	0	0	0	0

MeBCDmethanol	0	0	0	0	0	0	0
MeBCDphenol	0	0	0	0	0	0	0
MeBCDpropargylalcohol	0	0	0	0	0	0	0
MeBCDtertiarybutanol	0	0	0	0	0	0	0
PBS1-hexanol	0	0	0	0	0	0	0
PBSMCHM	0	0	0	0	0	0	0
PBSTHF	0	0	0	0	0	0	0
PBSbenzylalcohol	0	0	0	0	0	0	0
PBScyclohexanol	0	0	0	0	0	0	0
PBSethanol	0	0	0	0	0	0	0
PBSethyleneglycol	0	0	0	0	0	0	0
PBSmethanol	0	0	0	0	0	0	0
PBSphenol	0	0	0	0	0	0	0
PBSpropargylalcohol	0	0	0	0	0	0	0
PBStertiarybutanol	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	MeBCDtertiarybutanol	PBS1-hexanol	PBSMCHM	PBSTHF	PBSbenzylalcohol	PBScyclohexanol	PBSethanol	PBSethyleneglycol
2HPCD1-hexanol	0	0	0	0	0	0	0	0
2HPCDMCHM	0	0	0	0	0	0	0	0
2HPCDTHF	0	0	0	0	0	0	0	0
2HPCDbenzylalcohol	0	0	0	0	0	0	0	0
2HPCDcyclohexanol	0	0	0	0	0	0	0	0
2HPCDethanol	0	0	0	0	0	0	0	0
2HPCDethyleneglycol	0	0	0	0	0	0	0	0
2HPCDmethanol	0	0	0	0	0	0	0	0
2HPCDphenol	0	0	0	0	0	0	0	0
2HPCDpropargylalcohol	0	0	0	0	0	0	0	0
2HPCDtertiarybutanol	0	0	0	0	0	0	0	0
ACD1-hexanol	0	0	0	0	0	0	0	0
ACDMCHM	0	0	0	0	0	0	0	0
ACDTHF	0	0	0	0	0	0	0	0
ACDbenzylalcohol	0	0	0	0	0	0	0	0
ACDcyclohexanol	0	0	0	0	0	0	0	0
ACDethanol	0	0	0	0	0	0	0	0
ACDethyleneglycol	0	0	0	0	0	0	0	0
ACDmethanol	0	0	0	0	0	0	0	0
ACDphenol	0	0	0	0	0	0	0	0

ACDpropargylalcohol	0	0	0	0	0	0	0
ACDtertiarybutanol	0	0	0	0	0	0	0
BCD1-hexanol	0	0	0	0	0	0	0
BCDMCHM	0	0	1	0	0	0	0
BCDTHF	0	0	0	0	0	0	0
BCDbenzylalcohol	0	0	0	0	0	0	0
BCDcyclohexanol	0	0	0	0	0	0	0
BCDethanol	0	0	0	0	0	0	0
BCDethyleneglycol	0	0	0	0	0	0	0
BCDmethanol	0	0	0	0	0	0	0
BCDphenol	0	0	0	0	0	0	0
BCDpropargylalcohol	0	0	0	0	0	0	0
BCDtertiarybutanol	0	0	0	0	0	0	0
MeBCD1-hexanol	0	0	0	0	0	0	0
MeBCDMCHM	0	0	0	0	0	0	0
MeBCDTHF	0	0	0	0	0	0	0
MeBCDbenzylalcohol	0	0	0	0	0	0	0
MeBCDcyclohexanol	0	0	0	0	0	0	0
MeBCDethanol	0	0	0	0	0	0	0
MeBCDethyleneglycol	0	0	0	0	0	0	0
MeBCDmethanol	0	0	0	0	0	0	0
MeBCDphenol	0	0	0	0	0	0	0

Jackknifed Classification Matrix (Contd.)

	PBSmethanol	PBSphenol	PBSpropargylalcohol	PBStertiarybutanol	%correct
2HPCD1-hexanol	0	0	0	0	100
2HPCDMCHM	0	0	0	0	100
2HPCDTHF	0	0	0	0	100
2HPCDbenzylalcohol	0	0	0	0	100
2HPCDcyclohexanol	0	0	0	0	100
2HPCDethanol	0	0	0	0	100
2HPCDethyleneglycol	0	0	0	0	100
2HPCDmethanol	0	0	0	0	100
2HPCDphenol	0	0	0	0	100
2HPCDpropargylalcohol	0	0	0	0	100
2HPCDtertiarybutanol	0	0	0	0	100
ACD1-hexanol	0	0	0	0	100
ACDMCHM	0	0	0	0	100
ACDTHF	0	0	0	0	100
ACDbenzylalcohol	0	0	0	0	100
ACDcyclohexanol	0	0	0	0	100
ACDethanol	0	0	0	0	100
ACDethyleneglycol	0	0	0	0	100

ACDmethanol	0	0	0	0	100
ACDphenol	0	0	0	0	100
ACDpropargylalcohol	0	0	0	0	100
ACDtertiarybutanol	0	0	0	0	100
BCD1-hexanol	0	0	0	0	100
BCDMCHM	0	0	0	0	75
BCDTHF	0	0	0	0	100
BCDbenzylalcohol	0	0	0	0	100
BCDcyclohexanol	0	0	0	0	100
BCDethanol	0	0	0	0	100
BCDethyleneglycol	0	0	0	0	100
BCDmethanol	0	0	0	0	100
BCDphenol	0	0	0	0	100
BCDpropargylalcohol	0	0	0	0	100
BCDtertiarybutanol	0	0	0	0	100
MeBCD1-hexanol	0	0	0	0	100
MeBCDMCHM	0	0	0	0	100
MeBCDTHF	0	0	0	0	100
MeBCDbenzylalcohol	0	0	0	0	100
MeBCDcyclohexanol	0	0	0	0	100
MeBCDethanol	0	0	0	0	100
MeBCDethyleneglycol	0	0	0	0	100

MeBCDmethanol	0	0	0	0	100
MeBCDphenol	0	0	0	0	100
MeBCDpropargylalcohol	0	0	0	0	100
MeBCDtertiarybutanol	0	0	0	0	100
PBS1-hexanol	0	0	0	0	100
PBSMCHM	0	0	0	0	75
PBSTHF	0	0	0	0	100
PBSbenzylalcohol	0	0	0	0	100
PBScyclohexanol	0	0	0	0	100
PBSethanol	0	0	0	0	100
PBSethyleneglycol	0	0	0	0	100
PBSmethanol	4	0	0	0	100
PBSphenol	0	4	0	0	100
PBSpropargylalcohol	0	0	4	0	100
PBStertiarybutanol	0	0	0	4	100
Total	4	4	4	4	99

Cumulative Proportion of Total Dispersion

0.884	0.998	1.000
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Table S8. Results of array generation for analytes **1-13** with α -cyclodextrin with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	ACD1-hexanol	ACDMCHM	ACDTHF	ACDbenzylalcohol	ACDcyclohexanol	ACDethanol	ACDethyleneglycol	ACDmethanol
ACD1-hexanol	4	0	0	0	0	0	0	0
ACDMCHM	0	4	0	0	0	0	0	0
ACDTHF	0	0	4	0	0	0	0	0
ACDbenzylalcohol	0	0	0	4	0	0	0	0
ACDcyclohexanol	0	0	0	0	4	0	0	0
ACDethanol	0	0	0	0	0	4	0	0
ACDethyleneglycol	0	0	0	0	0	0	4	0
ACDmethanol	0	0	0	0	0	0	0	4
ACDphenol	0	0	0	0	0	0	0	0
ACDpropargylalcohol	0	0	0	0	0	0	0	0
ACDtertiarybutanol	0	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	ACDphenol	ACDpropargylalcohol	ACDtertiarybutanol	%correct
ACD1-hexanol	0	0	0	100
ACDMCHM	0	0	0	100
ACDTHF	0	0	0	100
ACDbenzylalcohol	0	0	0	100
ACDcyclohexanol	0	0	0	100
ACDethanol	0	0	0	100
ACDethyleneglycol	0	0	0	100
ACDmethanol	0	0	0	100
ACDphenol	4	0	0	100
ACDpropargylalcohol	0	4	0	100
ACDtertiarybutanol	0	0	4	100
Total	4	4	4	100

Cumulative Proportion of Total Dispersion

0.989	0.999	1.000
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Table S9. Results of array generation for analytes **1-13** with β -cyclodextrin with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	BCD1-hexanol	BCDMCHM	BCDTHF	BCDbenzylalcohol	BCDcyclohexanol	BCDethanol	BCDethyleneglycol	BCDmethanol
BCD1-hexanol	4	0	0	0	0	0	0	0
BCDMCHM	0	4	0	0	0	0	0	0
BCDTHF	0	0	4	0	0	0	0	0
BCDbenzylalcohol	0	0	0	4	0	0	0	0
BCDcyclohexanol	0	0	0	0	4	0	0	0
BCDethanol	0	0	0	0	0	4	0	0
BCDethyleneglycol	0	0	0	0	0	0	4	0
BCDmethanol	0	0	0	0	0	0	0	4
BCDphenol	0	0	0	0	0	0	0	0
BCDpropargylalcohol	0	0	0	0	0	0	0	0
BCDtertiarybutanol	0	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	BCDphenol	BCDpropargylalcohol	BCDtertiarybutanol	%correct
BCD1-hexanol	0	0	0	100
BCDMCHM	0	0	0	100
BCDTHF	0	0	0	100
BCDbenzylalcohol	0	0	0	100
BCDcyclohexanol	0	0	0	100
BCDethanol	0	0	0	100
BCDethyleneglycol	0	0	0	100
BCDmethanol	0	0	0	100
BCDphenol	4	0	0	100
BCDpropargylalcohol	0	4	0	100
BCDtertiarybutanol	0	0	4	100
Total	4	4	4	100

Cumulative Proportion of Total Dispersion

0.975	1.000	1.000
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Table S10. Results of array generation for analytes **1-13** with methyl- β -cyclodextrin with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	MeBCD1-hexanol	MeBCDMCHM	MeBCDTHF	MeBCDbenzylalcohol	MeBCDcyclohexanol	MeBCDethanol	MeBCDethyleneglycol
MeBCD1-hexanol	4	0	0	0	0	0	0
MeBCDMCHM	0	4	0	0	0	0	0
MeBCDTHF	0	0	4	0	0	0	0
MeBCDbenzylalcohol	0	0	0	4	0	0	0
MeBCDcyclohexanol	0	0	0	0	4	0	0
MeBCDethanol	0	0	0	0	0	4	0
MeBCDethyleneglycol	0	0	0	0	0	0	4
MeBCDmethanol	0	0	0	0	0	0	0
MeBCDphenol	0	0	0	0	0	0	0
MeBCDpropargylalcohol	0	0	0	0	0	0	0
MeBCDtertiarybutanol	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	MeBCDmethanol	MeBCDphenol	MeBCDpropargylalcohol	MeBCDtertiarybutanol	%correct
MeBCD1-hexanol	0	0	0	0	100
MeBCDMCHM	0	0	0	0	100
MeBCDTHF	0	0	0	0	100
MeBCDbenzylalcohol	0	0	0	0	100
MeBCDcyclohexanol	0	0	0	0	100
MeBCDethanol	0	0	0	0	100
MeBCDethyleneglycol	0	0	0	0	100
MeBCDmethanol	4	0	0	0	100
MeBCDphenol	0	4	0	0	100
MeBCDpropargylalcohol	0	0	4	0	100
MeBCDtertiarybutanol	0	0	0	4	100
Total	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.925	1.000	1.000
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Table S11. Results of array generation for analytes **1-13** with 2-hydroxypropyl- β -cyclodextrin with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	2HPCD1-hexanol	2HPCDMCHM	2HPCDTHF	2HPCDbenzylalcohol	2HPCDcyclohexanol	2HPCDethanol	2HPCDethyleneglycol
2HPCD1-hexanol	4	0	0	0	0	0	0
2HPCDMCHM	0	4	0	0	0	0	0
2HPCDTHF	0	0	4	0	0	0	0
2HPCDbenzylalcohol	0	0	0	4	0	0	0
2HPCDcyclohexanol	0	0	0	0	4	0	0
2HPCDethanol	0	0	0	0	0	4	0
2HPCDethyleneglycol	0	0	0	0	0	0	4
2HPCDmethanol	0	0	0	0	0	0	0
2HPCDphenol	0	0	0	0	0	0	0
2HPCDpropargylalcohol	0	0	0	0	0	0	0
2HPCDtertiarybutanol	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	2HPCDmethanol	2HPCDphenol	2HPCDpropargylalcohol	2HPCDtertiarybutanol	%correct
2HPCD1-hexanol	0	0	0	0	100
2HPCDMCHM	0	0	0	0	100
2HPCDTHF	0	0	0	0	100
2HPCDbenzylalcohol	0	0	0	0	100
2HPCDcyclohexanol	0	0	0	0	100
2HPCDethanol	0	0	0	0	100
2HPCDethyleneglycol	0	0	0	0	100
2HPCDmethanol	4	0	0	0	100
2HPCDphenol	0	4	0	0	100
2HPCDpropargylalcohol	0	0	4	0	100
2HPCDtertiarybutanol	0	0	0	4	100
Total	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.827	1.000	1.000
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Table S12. Results of array generation for analytes **1-13** with phosphate buffered saline with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	PBS1-hexanol	PBSMCHM	PBSTHF	PBSbenzylalcohol	PBScyclohexanol	PBSethanol	PBSethyleneglycol	PBSmethanol
PBS1-hexanol	4	0	0	0	0	0	0	0
PBSMCHM	0	4	0	0	0	0	0	0
PBSTHF	0	0	4	0	0	0	0	0
PBSbenzylalcohol	0	0	0	4	0	0	0	0
PBScyclohexanol	0	0	0	0	4	0	0	0
PBSethanol	0	0	0	0	0	4	0	0
PBSethyleneglycol	0	0	0	0	0	0	4	0
PBSmethanol	0	0	0	0	0	0	0	4
PBSphenol	0	0	0	0	0	0	0	0
PBSpropargylalcohol	0	0	0	0	0	0	0	0
PBSTertiarybutanol	0	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	PBSphenol	PBSpropargylalcohol	PBStertiarybutanol	%correct
PBS1-hexanol	0	0	0	100
PBSMCHM	0	0	0	100
PBSTHF	0	0	0	100
PBSbenzylalcohol	0	0	0	100
PBScyclohexanol	0	0	0	100
PBSethanol	0	0	0	100
PBSethyleneglycol	0	0	0	100
PBSmethanol	0	0	0	100
PBSphenol	4	0	0	100
PBSpropargylalcohol	0	4	0	100
PBStertiarybutanol	0	0	4	100
Total	4	4	4	100

Cumulative Proportion of Total Dispersion

0.984	0.994	1.000
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SUMMARY TABLES FOR MIXTURE ARRAY GENERATION

Table S13. Results of array generation for mixtures of analytes **2** and **8** with all cyclodextrin hosts with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	2HPCD 1:1 CH:MCHM	2HPCD 1:2 CH:MCHM	2HPCD 2:1 CH:MCHM	2HPCDMCHM	2HPCDcyclohexanol	ACD 1:1 CH:MCHM	ACD 1:2 CH:MCHM	ACD 2:1 CH:MCHM
2HPCD 1:1 CH:MCHM	4	0	0	0	0	0	0	0
2HPCD 1:2 CH:MCHM	0	4	0	0	0	0	0	0
2HPCD 2:1 CH:MCHM	0	0	4	0	0	0	0	0
2HPCDMCHM	0	0	0	4	0	0	0	0
2HPCDcyclohexanol	0	0	0	0	4	0	0	0
ACD 1:1 CH:MCHM	0	0	0	0	0	4	0	0
ACD 1:2 CH:MCHM	0	0	0	0	0	0	4	0
ACD 2:1 CH:MCHM	0	0	0	0	0	0	0	4
ACDMCHM	0	0	0	0	0	0	0	0
ACDcyclohexanol	0	0	0	0	0	0	0	0
BCD 1:1 CH:MCHM	0	0	0	0	0	0	0	0
BCD 1:2 CH:MCHM	0	0	0	0	0	0	0	0
BCD 2:1 CH:MCHM	0	0	0	0	0	0	0	0
BCDMCHM	0	0	0	0	0	0	0	0

BCDcyclohexanol	0	0	0	0	0	0	0	0
MeBCD 1:1 CH:MCHM	0	0	0	0	0	0	0	0
MeBCD 1:2 CH:MCHM	0	0	0	0	0	0	0	0
MeBCD 2:1 CH:MCHM	0	0	0	0	0	0	0	0
MeBCDMCHM	0	0	0	0	0	0	0	0
MeBCDcyclohexanol	0	0	0	0	0	0	0	0
PBS 1:1 CH:MCHM	0	0	0	0	0	0	0	0
PBS 1:2 CH:MCHM	0	0	0	0	0	0	0	0
PBS 2:1 CH:MCHM	0	0	0	0	0	0	0	0
PBSMCHM	0	0	0	0	0	0	0	0
PBScyclohexanol	0	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	ACDMCHM	ACDcyclohexanol	BCD 1:1 CH:MCHM	BCD 1:2 CH:MCHM	BCD 2:1 CH:MCHM	BCDMCHM	BCDcyclohexanol	MeBCD 1:1 CH:MCHM
2HPCD 1:1 CH:MCHM	0	0	0	0	0	0	0	0
2HPCD 1:2 CH:MCHM	0	0	0	0	0	0	0	0
2HPCD 2:1 CH:MCHM	0	0	0	0	0	0	0	0
2HPCDMCHM	0	0	0	0	0	0	0	0
2HPCDcyclohexanol	0	0	0	0	0	0	0	0
ACD 1:1 CH:MCHM	0	0	0	0	0	0	0	0
ACD 1:2 CH:MCHM	0	0	0	0	0	0	0	0
ACD 2:1 CH:MCHM	0	0	0	0	0	0	0	0
ACDMCHM	4	0	0	0	0	0	0	0
ACDcyclohexanol	0	4	0	0	0	0	0	0
BCD 1:1 CH:MCHM	0	0	4	0	0	0	0	0
BCD 1:2 CH:MCHM	0	0	0	4	0	0	0	0
BCD 2:1 CH:MCHM	0	0	0	0	4	0	0	0
BCDMCHM	0	0	0	0	0	3	0	0
BCDcyclohexanol	0	0	0	0	0	0	4	0

MeBCD 1:1 CH:MCHM	0	0	0	0	0	0	0	4
MeBCD 1:2 CH:MCHM	0	0	0	0	0	0	0	0
MeBCD 2:1 CH:MCHM	0	0	0	0	0	0	0	0
MeBCDMCHM	0	0	0	0	0	0	0	0
MeBCDcyclohexanol	0	0	0	0	0	0	0	0
PBS 1:1 CH:MCHM	0	0	0	0	0	0	0	0
PBS 1:2 CH:MCHM	0	0	0	0	0	0	0	0
PBS 2:1 CH:MCHM	0	0	0	0	0	0	0	0
PBSMCHM	0	0	0	0	0	1	0	0
PBScyclohexanol	0	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	MeBCD 1:2 CH:MCHM	MeBCD 2:1 CH:MCHM	MeBCDMCHM	MeBCDcyclohexan- ol	PBS 1:1 CH:MCHM	PBS 1:2 CH:MCHM	PBS 2:1 CH:MCHM	PBSMCHM
2HPCD 1:1 CH:MCHM	0	0	0	0	0	0	0	0
2HPCD 1:2 CH:MCHM	0	0	0	0	0	0	0	0
2HPCD 2:1 CH:MCHM	0	0	0	0	0	0	0	0
2HPCDMCHM	0	0	0	0	0	0	0	0
2HPCDcyclohexanol	0	0	0	0	0	0	0	0
ACD 1:1 CH:MCHM	0	0	0	0	0	0	0	0
ACD 1:2 CH:MCHM	0	0	0	0	0	0	0	0
ACD 2:1 CH:MCHM	0	0	0	0	0	0	0	0
ACDMCHM	0	0	0	0	0	0	0	0
ACDcyclohexanol	0	0	0	0	0	0	0	0
BCD 1:1 CH:MCHM	0	0	0	0	0	0	0	0
BCD 1:2 CH:MCHM	0	0	0	0	0	0	0	0
BCD 2:1 CH:MCHM	0	0	0	0	0	0	0	0
BCDMCHM	0	0	0	0	0	0	0	1
BCDcyclohexanol	0	0	0	0	0	0	0	0
MeBCD 1:1 CH:MCHM	0	0	0	0	0	0	0	0

MeBCD 1:2 CH:MCHM	4	0	0	0	0	0	0	0
MeBCD 2:1 CH:MCHM	1	3	0	0	0	0	0	0
MeBCDMCHM	0	0	4	0	0	0	0	0
MeBCDcyclohexanol	0	0	0	4	0	0	0	0
PBS 1:1 CH:MCHM	0	0	0	0	4	0	0	0
PBS 1:2 CH:MCHM	0	0	0	0	0	4	0	0
PBS 2:1 CH:MCHM	0	0	0	0	0	0	4	0
PBSMCHM	0	0	0	0	0	0	0	3
PBScyclohexanol	0	0	0	0	0	0	0	0
Total	5	3	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	PBScyclohexanol	%correct
2HPCD 1:1 CH:MCHM	0	100
2HPCD 1:2 CH:MCHM	0	100
2HPCD 2:1 CH:MCHM	0	100
2HPCDMCHM	0	100
2HPCDcyclohexanol	0	100
ACD 1:1 CH:MCHM	0	100
ACD 1:2 CH:MCHM	0	100
ACD 2:1 CH:MCHM	0	100
ACDMCHM	0	100
ACDcyclohexanol	0	100
BCD 1:1 CH:MCHM	0	100
BCD 1:2 CH:MCHM	0	100
BCD 2:1 CH:MCHM	0	100
BCDMCHM	0	75
BCDcyclohexanol	0	100

MeBCD 1:1 CH:MCHM	0	100
MeBCD 1:2 CH:MCHM	0	100
MeBCD 2:1 CH:MCHM	0	75
MeBCDMCHM	0	100
MeBCDcyclohexanol	0	100
PBS 1:1 CH:MCHM	0	100
PBS 1:2 CH:MCHM	0	100
PBS 2:1 CH:MCHM	0	100
PBSMCHM	0	75
PBScyclohexanol	4	100
Total	4	97

Cumulative Proportion of Total Dispersion

0.986	0.999	1.000
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Table S14. Results of array generation for mixtures of analytes **2** and **8** with α -cyclodextrin with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	ACD 1:1 CH:MCHM	ACD 1:2 CH:MCHM	ACD 2:1 CH:MCHM	ACDMCHM	ACDcyclohexanol	%correct
ACD 1:1 CH:MCHM	4	0	0	0	0	100
ACD 1:2 CH:MCHM	0	4	0	0	0	100
ACD 2:1 CH:MCHM	0	0	4	0	0	100
ACDMCHM	0	0	0	4	0	100
ACDcyclohexanol	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.998	1.000	1.000
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Table S15. Results of array generation for mixtures of analytes **2** and **8** with β -cyclodextrin with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	BCD 1:1 CH:MCHM	BCD 1:2 CH:MCHM	BCD 2:1 CH:MCHM	BCDMCHM	BCDcyclohexanol	%correct
BCD 1:1 CH:MCHM	4	0	0	0	0	100
BCD 1:2 CH:MCHM	0	4	0	0	0	100
BCD 2:1 CH:MCHM	0	0	4	0	0	100
BCDMCHM	0	0	0	4	0	100
BCDcyclohexanol	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

1.000	1.000	1.000
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Table S16. Results of array generation for mixtures of analytes **2** and **8** with methyl- β -cyclodextrin with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	MeBCD 1:1 CH:MCHM	MeBCD 1:2 CH:MCHM	MeBCD 2:1 CH:MCHM	MeBCDMCHM	MeBCDcyclohexanol	%correct
MeBCD 1:1 CH:MCHM	4	0	0	0	0	100
MeBCD 1:2 CH:MCHM	0	4	0	0	0	100
MeBCD 2:1 CH:MCHM	0	1	3	0	0	75
MeBCDMCHM	0	0	0	4	0	100
MeBCDcyclohexanol	0	0	0	0	4	100
Total	4	5	3	4	4	95

Cumulative Proportion of Total Dispersion

0.977	1.000	1.000
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Table S17. Results of array generation for mixtures of analytes **2** and **8** with 2-hydroxypropyl- β -cyclodextrin with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	2HPCD 1:1 CH:MCHM	2HPCD 1:2 CH:MCHM	2HPCD 2:1 CH:MCHM	2HPCDMCHM	2HPCDcyclohexanol	%correct
2HPCD 1:1 CH:MCHM	4	0	0	0	0	100
2HPCD 1:2 CH:MCHM	0	4	0	0	0	100
2HPCD 2:1 CH:MCHM	0	0	4	0	0	100
2HPCDMCHM	0	0	0	4	0	100
2HPCDcyclohexanol	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.996	1.000	1.000
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Table S18. Results of array generation for mixtures of analytes **2** and **8** with phosphate buffered saline with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	PBS 1:1 CH:MCHM	PBS 1:2 CH:MCHM	PBS 2:1 CH:MCHM	PBSMCHM	PBScyclohexanol	%correct
PBS 1:1 CH:MCHM	4	0	0	0	0	100
PBS 1:2 CH:MCHM	0	4	0	0	0	100
PBS 2:1 CH:MCHM	0	0	4	0	0	100
PBSMCHM	0	0	0	4	0	100
PBScyclohexanol	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.998	1.000	1.000
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Table S19. Results of array generation for analyte **8** in different solvent systems with β -cyclodextrin with fluorophores **14-16** as predictors

Jackknifed Classification Matrix

	1M NaCl MCHM	PB MCHM	PBS MCHM	PureWater MCHM	Seawater MCHM	%correct
1M NaCl MCHM	4	0	0	0	0	100
PB MCHM	0	4	0	0	0	100
PBS MCHM	0	0	4	0	0	100
PureWater MCHM	0	0	0	4	0	100
Seawater MCHM	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.955	1.000	1.000
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SUMMARY FIGURES FOR FLUORESCENCE MODULATION EXPERIMENTS

Figure S5. Fluorescence modulation of fluorophore 14 with analyte

Figure S6. Fluorescence modulation of fluorophore 15 with analyte 1

Figure S7. Fluorescence modulation of fluorophore 16 with analyte 1

Figure S8. Fluorescence modulation of fluorophore 14 with analyte 2

Figure S9. Fluorescence modulation of fluorophore **15** with analyte **2**

Figure S10. Fluorescence modulation of fluorophore **16** with analyte **2**

Figure S11. Fluorescence modulation of fluorophore 14 with analyte 3

Figure S12. Fluorescence modulation of fluorophore 15 with analyte 3

Figure S13. Fluorescence modulation of fluorophore 16 with analyte 3

Figure S14. Fluorescence modulation of fluorophore 14 with analyte 4

Figure S15. Fluorescence modulation of fluorophore **15** with analyte **4**

Figure S16. Fluorescence modulation of fluorophore **16** with analyte **4**

Figure S17. Fluorescence modulation of fluorophore **14** with analyte **5**

Figure S18. Fluorescence modulation of fluorophore **15** with analyte **5**

Figure S19. Fluorescence modulation of fluorophore **16** with analyte **5**

Figure S20. Fluorescence modulation of fluorophore **14** with analyte **6**

Figure S21. Fluorescence modulation of fluorophore **15** with analyte **6**

Figure S22. Fluorescence modulation of fluorophore **16** with analyte **6**

Figure S23. Fluorescence modulation of fluorophore **14** with analyte **7**

Figure S24. Fluorescence modulation of fluorophore **15** with analyte **7**

Figure S25. Fluorescence modulation of fluorophore 16 with analyte 7

Figure S26. Fluorescence modulation of fluorophore 14 with analyte 8

Figure S27. Fluorescence modulation of fluorophore **15** with analyte **8**

Figure S28. Fluorescence modulation of fluorophore **16** with analyte **8**

Figure S29. Fluorescence modulation of fluorophore **14** with analyte **9**

Figure S30. Fluorescence modulation of fluorophore **15** with analyte **9**

Figure S31. Fluorescence modulation of fluorophore 16 with analyte 9

Figure S32. Fluorescence modulation of fluorophore 14 with analyte 10

Figure S33. Fluorescence modulation of fluorophore **15** with analyte **10**

Figure S34. Fluorescence modulation of fluorophore **16** with analyte **10**

Figure S35. Fluorescence modulation of fluorophore **14** with analyte **11**

Figure S36. Fluorescence modulation of fluorophore **15** with analyte **11**

Figure S37. Fluorescence modulation of fluorophore 16 with analyte 11

Figure S38. Fluorescence modulation of fluorophore 14 with analyte 12

Figure S39. Fluorescence modulation of fluorophore 15 with analyte 12

Figure S40. Fluorescence modulation of fluorophore 16 with analyte 12

Figure S41. Fluorescence modulation of fluorophore **14** with analyte **13**

Figure S42. Fluorescence modulation of fluorophore **15** with analyte **13**

Figure S43. Fluorescence modulation of fluorophore **16** with analyte **13**

FLUORESCENCE MODULATION FIGURES FOR ANALYTE MIXTURES

Figure S44. Fluorescence modulation of fluorophore **14** with a 2:1 (vol:vol) mixture of analytes **2** and **8**

Figure S45. Fluorescence modulation of fluorophore **15** with a 2:1 (vol:vol) mixture of analytes **2** and **8**

Figure S46. Fluorescence modulation of fluorophore **16** with a 2:1 (vol:vol) mixture of analytes **2** and **8**

Figure S47. Fluorescence modulation of fluorophore **14** with a 1:1 (vol:vol) mixture of analytes **2** and **8**

Figure S48. Fluorescence modulation of fluorophore **15** with a 1:1 (vol:vol) mixture of analytes **2** and **8**

Figure S49. Fluorescence modulation of fluorophore **16** with a 1:1 (vol:vol) mixture of analytes **2** and **8**

Figure S50. Fluorescence modulation of fluorophore **14** with a 1:2 (vol:vol) mixture of analytes **2** and **8**

Figure S51. Fluorescence modulation of fluorophore **15** with a 1:2 (vol:vol) mixture of analytes **2** and **8**

Figure S52. Fluorescence modulation of fluorophore **16** with a 1:2 (vol:vol) mixture of analytes **2** and **8**

FLUORESCENCE EXPERIMENTS WITH DIFFERENT SOLVENT SYSTEMS

Figure S53. Fluorescence modulation of fluorophore **14** with analyte **8** with β -cyclodextrin in different solvent systems

Figure S54. Fluorescence modulation of fluorophore **15** with analyte **8** with β -cyclodextrin in different solvent systems

Figure S55. Fluorescence modulation of fluorophore **16** with analyte **8** with β -cyclodextrin in different solvent systems

SUMMARY FIGURES FOR ARRAY GENERATION EXPERIMENTS

Figure S56. Linear discriminant analysis of fluorescence responses for all analyte-fluorophore-host combinations with fluorophores **14-16** as predictors

Figure S57. Linear discriminant analysis of fluorescence responses for analytes **1-13** with α -cyclodextrin with fluorophores **14-16** as predictors

Figure S58. Linear discriminant analysis of fluorescence responses for analytes **1-13** with β -cyclodextrin with fluorophores **14-16** as predictors

Figure S59. Linear discriminant analysis of fluorescence responses for analytes **1-13** with methyl- β -cyclodextrin with fluorophores **14-16** as predictors

Figure S60. Linear discriminant analysis of fluorescence responses for analytes **1-13** with 2-hydroxypropyl- β -cyclodextrin with fluorophores **14-16** as predictors

Figure S61. Linear discriminant analysis of fluorescence responses for analytes **1-13** with phosphate buffered saline with fluorophores **14-16** as predictors

Figure S62. Linear discriminant analysis of fluorescence responses for mixtures of analytes **2** and **8** with all cyclodextrin hosts with fluorophores **14-16** as predictors

Figure S63. Linear discriminant analysis of fluorescence responses for mixtures of analytes **2** and **8** with α -cyclodextrin with fluorophores **14-16** as predictors

Figure S64. Linear discriminant analysis of fluorescence responses for mixtures of analytes **2** and **8** with β -cyclodextrin with fluorophores **14-16** as predictors

Figure S65. Linear discriminant analysis of fluorescence responses for mixtures of analytes **2** and **8** with methyl- β -cyclodextrin with fluorophores **14-16** as predictors

Figure S66. Linear discriminant analysis of fluorescence responses for mixtures of analytes **2** and **8** with 2-hydroxypropyl- β -cyclodextrin with fluorophores **14-16** as predictors

Figure S67. Linear discriminant analysis of fluorescence responses for mixtures of analytes **2** and **8** with phosphate buffered saline with fluorophores **14-16** as predictors

Figure S68. Linear discriminant analysis of fluorescence responses for analyte **8** in different solvent systems with fluorophores **14-16** as predictors

SUMMARY FIGURES FOR LIMIT OF DETECTION EXPERIMENTS

Figure S69. Limit of detection of analyte **1** with fluorophore **14** with α -cyclodextrin

Figure S70. Limit of detection of analyte **1** with fluorophore **14** with β -cyclodextrin

Figure S71. Limit of detection of analyte **1** with fluorophore **14** with methyl- β -cyclodextrin

Figure S72. Limit of detection of analyte **1** with fluorophore **14** with 2-hydroxypropyl- β -cyclodextrin

Figure S73. Limit of detection of analyte **1** with fluorophore **14** with phosphate buffered saline

Figure S74. Limit of detection of analyte **2** with fluorophore **14** with α -cyclodextrin

Figure S75. Limit of detection of analyte **2** with fluorophore **14** with β -cyclodextrin

Figure S76. Limit of detection of analyte **2** with fluorophore **14** with methyl- β -cyclodextrin

Figure S77. Limit of detection of analyte **2** with fluorophore **14** with 2-hydroxypropyl- β -cyclodextrin

Figure S78. Limit of detection of analyte **2** with fluorophore **14** with phosphate buffered saline

Figure S79. Limit of detection of analyte **3** with fluorophore **14** with α -cyclodextrin

Figure S80. Limit of detection of analyte **3** with fluorophore **14** with β -cyclodextrin

Figure S81. Limit of detection of analyte **3** with fluorophore **14** with methyl- β -cyclodextrin

Figure S82. Limit of detection of analyte **3** with fluorophore **14** with 2-hydroxypropyl- β -cyclodextrin

Figure S83. Limit of detection of analyte **3** with fluorophore **14** with phosphate buffered saline

Figure S84. Limit of detection of analyte **3** with fluorophore **15** with α -cyclodextrin

Figure S85. Limit of detection of analyte **3** with fluorophore **15** with β -cyclodextrin

Figure S86. Limit of detection of analyte **3** with fluorophore **15** with methyl- β -cyclodextrin

Figure S87. Limit of detection of analyte **3** with fluorophore **15** with 2-hydroxypropyl- β -cyclodextrin

Figure S88. Limit of detection of analyte **3** with fluorophore **15** with phosphate buffered saline

Figure S89. Limit of detection of analyte **3** with fluorophore **16** with α -cyclodextrin

Figure S90. Limit of detection of analyte **3** with fluorophore **16** with β -cyclodextrin

Figure S91. Limit of detection of analyte **3** with fluorophore **16** with methyl- β -cyclodextrin

Figure S92. Limit of detection of analyte **3** with fluorophore **16** with 2-hydroxypropyl- β -cyclodextrin

Figure S93. Limit of detection of analyte **3** with fluorophore **16** with phosphate buffered saline

Figure S94. Limit of detection of analyte **4** with fluorophore **14** with α -cyclodextrin

Figure S95. Limit of detection of analyte **4** with fluorophore **14** with β -cyclodextrin

Figure S96. Limit of detection of analyte **4** with fluorophore **14** with methyl- β -cyclodextrin

Figure S97. Limit of detection of analyte **4** with fluorophore **14** with 2-hydroxypropyl- β -cyclodextrin

Figure S98. Limit of detection of analyte **4** with fluorophore **14** with phosphate buffered saline

Figure S99. Limit of detection of analyte **4** with fluorophore **15** with α -cyclodextrin

Figure S100. Limit of detection of analyte **4** with fluorophore **15** with β -cyclodextrin

Figure S101. Limit of detection of analyte **4** with fluorophore **15** with methyl-β-cyclodextrin

Figure S102. Limit of detection of analyte **4** with fluorophore **15** with 2-hydroxypropyl-β-cyclodextrin

Figure S103. Limit of detection of analyte **4** with fluorophore **15** with phosphate buffered saline

Figure S104. Limit of detection of analyte **4** with fluorophore **16** with α -cyclodextrin

Figure S105. Limit of detection of analyte **4** with fluorophore **16** with β -cyclodextrin

Figure S106. Limit of detection of analyte **4** with fluorophore **16** with methyl- β -cyclodextrin

Figure S107. Limit of detection of analyte **4** with fluorophore **16** with 2-hydroxypropyl- β -cyclodextrin

Figure S108. Limit of detection of analyte **4** with fluorophore **16** with phosphate buffered saline

Figure S109. Limit of detection of analyte **8** with fluorophore **14** with α -cyclodextrin

Figure S110. Limit of detection of analyte **8** with fluorophore **14** with β -cyclodextrin

Figure S111. Limit of detection of analyte **8** with fluorophore **14** with methyl- β -cyclodextrin

Figure S112. Limit of detection of analyte **8** with fluorophore **14** with 2-hydroxypropyl- β -cyclodextrin

Figure S113. Limit of detection of analyte **8** with fluorophore **14** with phosphate buffered saline

Figure S114. Limit of detection of analyte **9** with fluorophore **14** with α -cyclodextrin

Figure S115. Limit of detection of analyte **9** with fluorophore **14** with β -cyclodextrin

Figure S116. Limit of detection of analyte **9** with fluorophore **14** with methyl- β -cyclodextrin

Figure S117. Limit of detection of analyte **9** with fluorophore **14** with 2-hydroxypropyl- β -cyclodextrin

Figure S118. Limit of detection of analyte **9** with fluorophore **14** with phosphate buffered saline

Figure S119. Limit of detection of analyte **10** with fluorophore **14** with α -cyclodextrin

Figure S120. Limit of detection of analyte **10** with fluorophore **14** with β -cyclodextrin

Figure S121. Limit of detection of analyte **10** with fluorophore **14** with methyl- β -cyclodextrin

Figure S122. Limit of detection of analyte **10** with fluorophore **14** with 2-hydroxypropyl- β -cyclodextrin

Figure S123. Limit of detection of analyte **10** with fluorophore **14** with phosphate buffered saline

Figure S124. Limit of detection of analyte **11** with fluorophore **14** with α -cyclodextrin

Figure S125. Limit of detection of analyte **11** with fluorophore **14** with β -cyclodextrin

Figure S126. Limit of detection of analyte **11** with fluorophore **14** with methyl- β -cyclodextrin

Figure S127. Limit of detection of analyte **11** with fluorophore **14** with 2-hydroxypropyl- β -cyclodextrin

Figure S128. Limit of detection of analyte **11** with fluorophore **14** with phosphate buffered saline

Figure S129. Limit of detection of analyte **12** with fluorophore **14** with α -cyclodextrin

Figure S130. Limit of detection of analyte **12** with fluorophore **14** with β -cyclodextrin

Figure S131. Limit of detection of analyte **12** with fluorophore **14** with methyl- β -cyclodextrin

Figure S132. Limit of detection of analyte **12** with fluorophore **14** with 2-hydroxypropyl- β -cyclodextrin

Figure S133. Limit of detection of analyte **12** with fluorophore **14** with phosphate buffered saline

CHAPTER 2

Published in *New Journal of Chemistry* **2016**, 40, 789-793

Selective Detection of Non-Aromatic Pesticides via Cyclodextrin-Promoted
Fluorescence Modulation

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Manuscript 2

Selective Detection of Non-Aromatic Pesticides via Cyclodextrin-Promoted Fluorescence Modulation

ABSTRACT

Reported herein is the detection of aliphatic pesticides using cyclodextrin-promoted fluorescence modulation. The introduction of the pesticide leads to noticeable changes in the emission of a fluorophore held in close proximity by cyclodextrin, leading to a pesticide detection system that operates with 100% successful differentiation and low limits of detection.

INTRODUCTION

The widespread use of pesticides is of significant concern due to the known and suspected toxicities of many small-molecule pesticides,¹ as well as their long-term environmental persistence.² Currently used methods for pesticide detection overwhelmingly rely on chromatography for the purification of the small molecule pesticides, followed by mass spectrometry for accurate pesticide identification.³ Although such methods are highly sensitive for individual pesticides, they tend to suffer from tedious sample preparations, which limits the ability to conduct high throughput assays of multiple samples for multiple pesticide analytes.⁴ Newer detection methods such as nanoparticle-based Raman spectroscopy⁵ and electrochemical assays⁶ have recently been developed.

The use of fluorescence-based methods for pesticide detection would be highly advantageous, as fluorescence spectroscopy is sensitive to even trace amounts of a target analyte,⁷ can rapidly generate response signals,⁸ and is amenable to the development of

high throughput assays for rapid screening of multiple environmental samples.⁹ Fluorescence assays for non-photophysically active compounds such as organochlorine pesticides (compounds **1-4**, Figure 1) are complicated by the fact that the analytes themselves do not have notable fluorescence signals, and require either derivatization¹⁰ or an indirect method for efficient fluorescence detection.¹¹

Figure 1. Pesticides (**1-4**), control analyte **5**, and fluorophores (**6-8**) under investigation

Previous research in our laboratory led to the development of cyclodextrin-promoted energy transfer for the detection of aromatic toxicants and toxicant metabolites¹² in multiple complex environments.¹³ This system uses the aromatic analytes as energy donors in combination with high quantum yield fluorophore acceptors, which are held in close proximity by the supramolecular cyclodextrin scaffold.

Recently, we have developed a new concept termed “fluorescence modulation,” which enables the detection of aliphatic analytes that do not participate in cyclodextrin-promoted energy transfer. These analytes are detectable through quantifying proximity-

induced changes in the fluorescence spectrum of a high quantum yield fluorophore upon introduction of the analyte (Figure 2), with the cyclodextrin acting as a supramolecular scaffold to ensure productive analyte-fluorophore interactions.

Figure 2. Schematic illustration of cyclodextrin-promoted fluorescence modulation with several potential association complexes

Reported herein is the use of such fluorescence modulation for the sensitive and selective detection of aliphatic pesticides **1-4**, with detection limits as low as 5.2 μM and 100% selectivity in array-based identification.

EXPERIMENTAL

Materials and Methods

All analytes and cyclodextrins were obtained from Sigma-Aldrich. Fluorophore **6** was synthesized according to literature procedures.¹⁷ All fluorescence measurements were performed using a Shimadzu RF 5301 spectrophotometer. All fluorescence spectra were integrated vs. wavenumber on the X-axis using OriginPro. All arrays were generated using SYSTAT.

Fluorescence Modulation Experiments

The fluorescence spectra were integrated vs. wavenumber on the X-axis, and fluorescence modulation was quantified using Equation 1.

Limit of Detection Experiments

The limit of detection (LOD) is defined as the lowest concentration of analyte at which a signal can be detected. The limit of quantification is defined at the lowest concentration of analyte that can be accurately quantified. These experiments were conducted following literature-reported procedures.⁵

Selection of Cyclodextrins

A variety of cyclodextrin scaffolds were investigated, including β -cyclodextrin (β -CD) and methyl- β -cyclodextrin (Me- β -CD) that are known to bind to small molecule fluorophores.¹⁴ Also included were 2-hydroxypropyl- β -cyclodextrin (2-HPCD), which has been shown to promote proximity-induced energy transfer,¹⁵ and α -cyclodextrin (α -CD), which is capable of acting as a supramolecular scaffold despite its limited cavity size.¹⁶ All results were compared to those obtained in a cyclodextrin-free phosphate buffered saline (PBS) solution, buffered at pH 7.4.

Selection of Fluorophores

The fluorophores chosen have all been investigated previously for their ability to participate in cyclodextrin-promoted energy transfer.¹² They are either commercially available (**7** and **8**) or were synthesized following literature-reported procedures.¹⁷

Quantification of Fluorescence Changes

The changes in the fluorophore emission spectra of fluorophores **6-8** with introduction of the pesticide analyte were determined by combining small amounts of fluorophores **6-8** in tetrahydrofuran with a 10 mM aqueous cyclodextrin solution. The fluorescence emission of the fluorophores in the cyclodextrin solution were compared

to their emission after the introduction of small amounts of pesticides **1-4** in tetrahydrofuran, and the results were quantified according to Equation 1:

$$\text{Fluorophore Ratio} = (Fl_{\text{analyte}})/Fl_{\text{blank}} \quad (\text{Eq. 1})$$

Where Fl_{analyte} is the fluorescence emission of the fluorophore in the presence of the analyte, and Fl_{blank} is the fluorescence emission of the fluorophore in the absence of the analyte.

RESULTS AND DISCUSSION

When combined with fluorophore **6** in α -CD, β -CD, or 2-HPCD, compounds **1-4** induced significant increases in the fluorophore's emission spectra (Table 1). In each of these cases, the changes in fluorescence emission were even greater in the absence of a CD host (indicated by higher fluorophore emission ratios in PBS).

Table 1. Selected fluorophore emission ratios with fluorophore **6**

Analyte	α -CD	β -CD	Me- β -CD	2-HPCD	PBS
1	1.12	1.15	1.01	1.10	1.22
2	1.15	1.15	0.97	1.14	1.33
3	1.30	1.24	0.96	1.18	1.27
4	1.26	1.15	0.99	1.08	1.34

These results suggest that pesticide-fluorophore interactions result in increases in the fluorophore emission of compound **6**, and that cyclodextrin hosts modulate the degree of fluorescence enhancement by partially shielding the fluorophore from such close interactions with the pesticide analytes. This hypothesis is supported by the limited fluorescence changes observed in Me- β -CD systems, which we have previously shown to be the most efficient cyclodextrin for the binding and extraction of fluorophore **6**.¹³

Moreover, a closer examination of the absolute fluorescence intensities in each of the cyclodextrin hosts (Figure 3) indicates that the fluorescence emission of compound **6** in methyl- β -cyclodextrin was markedly higher than its emission in any of

the other hosts. Increases in absolute fluorescence intensity generally indicate strong binding in a supramolecular host (due to the elimination of non-radiative decay pathways),¹⁸ and support the hypothesis that strong binding of the fluorophore in the cyclodextrin (in this case fluorophore **6** in Me- β -CD) limits its intermolecular interactions with the pesticide analyte.

Figure 3. Fluorescence changes in fluorophore **6** upon introduction of: (A) analyte **1**; (B) analyte **2**; (C) analyte **3**; and (D) analyte **4**. ($\lambda_{\text{ex}} = 460 \text{ nm}$)

Fluorophore **7** showed no significant changes in its fluorescence emission when combined with analytes **1** and **2** (Table 2). Analytes **3** and **4**, by contrast, led to noticeable changes in the fluorescence emission of fluorophore **7** in the presence of α -CD, β -CD, 2-HPCD and in the absence of any CD. In the presence of Me- β -CD, no significant changes in fluorophore **7**'s emission spectrum was observed, in line with the results observed for fluorophore **6** and in good agreement with our previously reported results.¹³

Table 2. Selected fluorophore emission ratios with fluorophore **7**

Analyte	α -CD	β -CD	Me- β -CD	2-HPCD	PBS
1	1.09	1.00	1.00	1.02	1.00
2	0.98	1.00	0.99	0.97	1.00
3	1.08	0.96	1.04	0.99	0.88
4	1.28	1.05	1.03	0.99	1.09

Fluorophore **8** showed an increase in fluorescence emission when combined with analytes **1-4** in the presence of α -CD; decrease in the fluorescence emission when combined with analytes **1-4** in the presence of Me- β -CD and 2-HPCD, and variable, analyte-dependent fluorescence changes when combined with analytes **1-4** in the presence of β -CD (Table 3).

Table 3. Selected fluorophore emission ratios with fluorophore **8**

Analyte	α -CD	β -CD	Me- β -CD	2-HPCD	PBS
1	1.21	1.01	0.97	0.88	1.22
2	1.07	1.03	0.87	0.94	1.10
3	1.08	1.24	0.94	0.88	1.23
4	1.21	1.08	0.83	0.94	1.23

Uniform increases in fluorescence were observed in the absence of any CD, which supports the notion of pesticide-mediated fluorescence increases that are being attenuated in the presence of favorably interacting cyclodextrin hosts.

Mechanism of Differentiation

Each component of the system has structural features that facilitate intermolecular interactions: (a) Cyclodextrins bind small molecule guests using a range of intermolecular forces, including hydrophobic association, hydrogen bonding, and van der Waals interactions.¹⁹ (b) Fluorophores **6-8** can participate in intermolecular interactions through aromatic π -stacking,²⁰ hydrophobic association and hydrogen bonding. (c) Pesticides **1-4** are expected to participate in intermolecular interactions

largely through hydrophobic association and/or through intermolecular interactions with the chlorine moieties.²¹

Figure 4. Electrostatic potential surfaces of analytes **1-4**, calculated using Spartan 10, version 1.1.0. The red color represents the electron-rich regions and the blue color represents electron-deficient regions

Electrostatic potential surfaces of pesticides **1-4** are shown in Figure 4 and quantitative data is summarized in Table 4. These marked differences in solvent accessible surface area and in electrostatic potential surfaces contributes to the differences in the interactions of these analytes with fluorophores and cyclodextrins, which gives rise to the unique fluorescence modulation patterns. These patterns enable high levels of selectivity in array-based detection (*vide infra*), even though the quantitative binding constants of the analytes in β -cyclodextrin are relatively weak (Table 4).

Table 4. Quantitative information about the small molecule pesticides

Analyte	Binding in β -CD (mM ⁻¹) ^a	Solvent-accessible surface area (Å ²) ^c	Electrostatic potential (min/max) (kJ/mol) ^c
1	0.067	171	-50/102
2	^b	148	-118/100
3	0.067	160	-75/150
4	0.017	139	-96/174

^a Calculated by ¹H NMR titrations ^b ¹H NMR titrations indicated no measurable binding ^c Calculated using Spartan 10, version 1.1.0.

Overall, the low degrees of fluorescence modulation and weak binding affinities observed in these systems indicate the possibility that variable binding geometries may

exist, including those in which the pesticide and fluorophore are each bound to a different cyclodextrin molecule (Figure 2).

Literature precedent shows that halogen atoms interact with planar aromatic surfaces under a variety of conditions.²² In our system, we observe a similar degree of intermolecular interactions between the halogenated pesticides **1-4** and the aromatic surface areas of fluorophores **6-8** in the cyclodextrin-free solution, which results in quantifiable enhancements in the fluorescence emission spectra. In the presence of a variety of cyclodextrin hosts, the fluorophores preferentially bind to the cyclodextrin (K_a in β -CD = 0.11 mM⁻¹ for fluorophore **7**; 0.30 mM⁻¹ for fluorophore **8**). In such cases, the introduction of the pesticide analyte leads to attenuated interactions between the pesticide and the fluorophore, resulting in generally lower degrees of fluorescence enhancements.

Array-Based Detection

Overall, the observed fluorescence modulation responses are unique to each analyte-fluorophore-cyclodextrin combination, with the reported modulation providing a snapshot of the complicated interplay of intermolecular interactions between the three components. These unique responses generate well-separated signals for all of the pesticides investigated using linear discriminant analysis (LDA) methods (Figure 5),²³ with even a single cyclodextrin host providing sufficient differentiation for all target analytes.

Figure 5. Selected array results with hosts: (A) α -CD; (B) β -CD; (C) Me- β -CD; and (D) PBS.

Interestingly, these responses are markedly different than the response pattern obtained using tetrahydrofuran **5** as a control, which confirms that the fluorescence modulation is due to pesticide-specific interactions and is not a result of the small amount of tetrahydrofuran used to dissolve the pesticide analyte prior to addition.

Control experiments in the absence of any cyclodextrin (Figure 5D) indicate well-separated signals for the analytes; however, the scale of the X-axis indicates a markedly lower degree of separation in PBS and in α -cyclodextrin compared to the results obtained in β -cyclodextrin and Me- β -cyclodextrin, which highlights stronger intermolecular interactions with the larger cyclodextrin systems compared to the results obtained with the smaller α -cyclodextrin or without a cyclodextrin host.

The limits of detection (LODs) and limits of quantification (LOQs) of cyclodextrin-promoted fluorescence modulation were assayed using literature-reported methods,⁵ and some select results are highlighted in Table 5.

Table 5. Limits of detection calculated for analytes **1-4** with fluorophore **6** in β -CD and in PBS

Analyte	in β -CD	in PBS
1	82 μ M	<i>a</i>
2	<i>a</i>	<i>a</i>
3	31 μ M	<i>a</i>
4	189 μ M	5 μ M

^a Efforts to calculate limits of detection for these analyte-fluorophore-cyclodextrin combinations led to negative calculated values; current efforts are focused on understanding these phenomena

Detection limits as low as 5 μ M were obtained. These results are slightly higher than literature reported values for other pesticide detection systems; however, the system reported herein has a number of notable operational advantages that make it attractive from a practical detection perspective.²⁴ The negative calculated values occur because the limit of detection calculations use the assumption of a linear relationship between the fluorescence emission and the analyte concentration. Because the fluorescence emission in this case is a result of complicated intermolecular interactions between the analyte, fluorophore, and cyclodextrin, there are cases where that linear relationship does not hold.

The practicality of this system would be markedly enhanced by its ability to detect a broader range of lipophilic compounds with high sensitivity. Aromatic organochlorine pesticides have already been detected by our group using cyclodextrin-promoted energy transfer to fluorophores **6-8**,²⁵ and a variety of aliphatic alcohols have been detected via cyclodextrin-promoted fluorescence modulation (Table 6).²⁶

Table 6. Selected limits of detection for other small molecule analytes^a

Analyte	Limits of detection
bifenthrin	5 μM^b
cyfluthrin	17 μM^b
methanol	352 μM^c
ethanol	183 μM^c
1-hexanol	109 μM^c
cyclohexanol	22 μM^c

^a Data is from references 25 and 26 ^b Using γ -CD and fluorophore **7** ^c Using 2-HPCD and fluorophore **6**

In general, the limits of detection for the alkanols are higher than those of the aliphatic organochlorine pesticides, highlighting a lower degree of sensitivity for these other analytes.

CONCLUSION

In summary, measurable changes in fluorescence emission spectra are seen upon addition of an aliphatic analyte to a high quantum yield fluorophore. The degree of intermolecular interactions between the two components strongly depends on the structures of each component, as well as on the supramolecular host. These changes, termed “fluorescence modulation,” enable the rapid, sensitive, and selective detection of analytes such as aliphatic organochlorine pesticides, with 100% success in identifying unique response patterns for each analyte-CD-fluorophore combination and low limits of detection. The use of this fluorescence modulation technique for aliphatic alcohol detection in complex mixtures represents a significant challenge in terms of selectivity and sensitivity, and current efforts in our laboratory are focused on addressing these challenges and optimizing detection in complex environments. Results of these and other investigations will be reported in due course.

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**Selective Detection of Non-Aromatic Pesticides via Cyclodextrin-Promoted
Fluorescence Modulation**

MATERIALS AND METHODS

All analytes and cyclodextrins were obtained from Sigma-Aldrich chemical company. Fluorophore **6**, BODIPY, was synthesized according to literature procedures. All fluorescence measurements were performed using a Shimadzu RF 5301 spectrophotometer. Both the excitation slit width and the emission slit width were 1.5 nm. All fluorescence spectra were integrated vs. wavenumber on the X-axis using OriginPro Version 9.1. All arrays were generated using SYSTAT Version 13.

ANALYTE DETAILS

Figure S1. Structures of analytes **1-4**, control analyte **5**, and fluorophores **6-8**

FINAL SOLUTION CONCENTRATIONS

Table S1. Final analyte concentrations

Analyte Number	Amount Added	Final Concentration
1	20 μ L	14.1 μ M
2	20 μ L	18.8 μ M
3	20 μ L	20.6 μ M
4	20 μ L	26.4 μ M
5	20 μ L	106 μ M

Table S2. Final fluorophore concentrations

Fluorophore Number	Amount Added	Final Concentration
6	100 μ L	9.5 μ M
7	100 μ L	8.1 μ M
8	100 μ L	11 μ M

SYNTHESIS OF FLUOROPHORE 6

The synthesis of BODIPY **6** was performed according to literature procedures:

REFERENCE: Shepherd, J. L.; Kell, A.; Chung, E.; Sinclair, C. W.; Workentin, M. S.; Bizzotto, D. *J. Am. Chem. Soc.* **2004**, *126*, 8329-8335.

Reaction 1:

Figure S2. Synthetic pathway to **S5**

Procedure: 2.0 grams of 11-bromoundecanoic acid **S1** (7.54 mmol, 1.0 eq.) was combined with 2 drops of *N,N*-dimethylformamide in 40 mL of dichloromethane. 1.0 gram of oxalyl chloride **S2** (7.88 mmol, 1.05 eq.) was dissolved in 5.0 mL of dichloromethane and added dropwise. The reaction mixture was stirred for one hour, then the crude mixture was concentrated on the rotary evaporator and dried on a vacuum overnight to remove any unreacted oxalyl chloride. The resulting acid chloride **S3** was dissolved in 50 mL of dichloromethane. 0.772 mL of 2,4-dimethylpyrrole **S4** (7.50 mmol, 0.99 eq.) was dissolved in 5.0 mL of dichloromethane and added to the reaction mixture. The resulting reaction mixture was heated to reflux for 3 hours under a nitrogen atmosphere, during which time the mixture became a dark red color. After three hours, the reaction mixture was cooled to room temperature and solvent was removed on the rotary evaporator until approximately 5.0 mL of the dichloromethane solution remained. 200 mL of *n*-hexanes were added to the flask, and the mixture was cooled overnight in

the freezer at -20 °C. The hexanes were decanted from the insoluble oil and precipitate. The resulting crude product was dissolved in 75 mL of toluene and heated to 80 °C. 1.0 mL of triethylamine (7.17 mmol, 0.95 eq.) was added and the solution immediately turned light yellow. 1.0 mL of boron trifluoride etherate (8.10 mmol, 1.07 eq.) was then added and the reaction mixture was stirred at 80 °C for 30 minutes, during which time the color of the mixture darkened and became fluorescent. The reaction mixture was cooled to room temperature, and the product was extracted 3 times with brine (50 mL each time). The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography (1:1 dichloromethane: hexanes) to yield the desired product in 28% yield (comparable to the literature-reported 24% yield).

Reaction 2:

Figure S3. Synthetic pathway to **S7**

Procedure: Compound **S5** (0.968 g, 2.07 mmol, 1.0 eq.) and compound **S6** (0.27 grams, 2.36 mmol, 1.14 eq.) were dissolved in 50 mL of acetone. The reaction mixture was heated to reflux for two hours. After two hours, the reaction mixture was cooled to room temperature, acetone was removed, and the crude solid was re-dissolved in dichloromethane and washed with water. The organic extract was dried over sodium sulfate, filtered and concentrated, to yield compound **S7** in 97% yield (0.932 grams).

Reaction 3:

Figure S4. Synthetic pathway to BODIPY **6**

Procedure: Compound **S7** (0.932 grams, 2.01 mmol, 1.0 eq.) was dissolved in 150 mL of anhydrous ethanol that was purged with nitrogen. Potassium carbonate was added, and the reaction mixture was warmed to 30 °C. The reaction mixture was stirred under nitrogen for 4 hours at 30 °C. The contents of the flask were poured over 40 mL of aqueous saturated ammonium chloride, at which point the solution turned bright orange. The product was extracted with dichloromethane and washed several times with water. The organic layer was dried over sodium sulfate, filtered, and concentrated. The product was purified via flash chromatography (1:1 dichloromethane: hexanes) to yield compound **6** in 76% yield (674 mg).

EXPERIMENTAL DETAILS FOR FLUORESCENCE MODULATION EXPERIMENTS

In a quartz cuvette, 2.5 mL of a 10 mM cyclodextrin solution and 100 μ L of a solution of fluorophore **6** (0.1 mg/mL) were combined. The solution was excited at 460 nm, which is the excitation wavelength to directly excite compound **6**. 20 μ L of analyte solution (1 mg/mL) was added to the cuvette, and fluorescence measurements were repeated at 460 nm. The experiments were repeated for fluorophore **7** and **8** at excitation wavelengths of 490 nm and 420 nm, respectively. The fluorescence spectra were integrated vs. wavenumber on the X-axis, and fluorescence modulation was quantified using Equation 1:

$$\text{Fluorophore Ratio} = (Fl_{\text{analyte}})/Fl_{\text{blank}} \quad (\text{Equation 1})$$

where Fl_{analyte} is the fluorescence emission of the fluorophore in the presence of the analyte, and Fl_{blank} is the fluorescence emission of the fluorophore in the absence of the analyte. Each experiment was repeated four times. The reported values are the average of the results.

EXPERIMENTAL DETAILS FOR LIMIT OF DETECTION EXPERIMENTS

The limit of detection (LOD) is defined as the lowest concentration of analyte at which a signal can be detected. The limit of quantification is defined at the lowest concentration of analyte that can be accurately quantified. These experiments were conducted following literature-reported procedures:

REFERENCE: Saute, B.; Premasiri, R.; Ziegler, L.; Narayanan, R. "Gold Nanorods as Surface Enhanced Raman Spectroscopy Substrates for Sensitive and Selective Detection of Ultra-Low Levels of Dithiocarbamate Pesticides." *Analyst* **2012**, *137*, 5082-5087.

To determine the limit of detection (LOD) and limit of quantification (LOQ), each fluorophore-analyte combination was examined in the following manner:

1. 2.5 mL of 10 mM γ -cyclodextrin in phosphate-buffered saline (PBS) was measured into a cuvette and 100 μ L of a fluorophore solution in THF was added. The solution was excited at the analyte's excitation wavelength (see table of wavelengths below) and the fluorescence emission spectrum was recorded. Six repeat measurements were made for the fluorescence emission spectra.
2. 20 μ L of a 1 mg/mL analyte solution in THF was added to the cuvette and the solution was again excited at the analyte excitation wavelength. Six repeat measurements were taken.
3. Step 2 was repeated for 40 μ L of analyte, 60 μ L of analyte, 80 μ L of analyte, and 100 μ L of analyte. In each case, the solution was excited at the analyte excitation wavelength and the fluorescence emission spectrum was recorded four times.
4. All fluorescence emission spectra were integrated vs. wavenumber, and we generated calibration curves with the analyte concentration on the X-axis (in μ M) and the

integrated fluorophore emission on the Y-axis. The curve was then fitted to a straight line and an equation for the line was determined.

5. For each case, the fluorophore with γ -cyclodextrin (before any analyte was added) was also excited at the excitation wavelength for the analyte, and the fluorescence emission spectrum was recorded (as per step 1). These measurements are referred to as the “blank.”

6. The limit of the blank is defined according to the following equation:

$$LoB_{LOD} = m_{blank} + 3(SD_{blank})$$

Where m is the mean of the blank integrations and SD is the standard deviation.

7. The limit of the blank was then entered into the equation determined in step 4 (for the y value), and the corresponding X value was determined. This value provided the LOD in μM .

8. The limit of quantification (LOQ) was calculated in a similar way to the limit of detection. First, the limit of the blank for quantification was determined according to the following equation:

$$LoB_{LOQ} = m_{blank} + 10(SD_{blank})$$

This value was entered into the equation determined in step 4 (for the y value), and the corresponding X value was determined to be the limit of quantification in μM .

EXPERIMENTAL DETAILS FOR FLUORESCENCE ARRAY EXPERIMENTS

Array analysis was performed using SYSTAT 13 statistical computing software with the following settings: (a) Classical Discriminant Analysis; (b) Grouping Variable: Analytes; (c) Predictors: Bodipy, Rhodamine 6G, Coumarin 6; (d) Long-Range Statistics: Mahal

Arrays were generated for: (a) all analyte-fluorophore-cyclodextrin combinations in one array; and (b) individual cyclodextrin hosts in separate arrays. The results are summarized in the tables.

EXPERIMENTAL DETAILS FOR ^1H NMR TITRATION EXPERIMENTS

A solution of 10 mM β -CD was prepared in deuterium oxide. In an NMR tube, the β -CD and varying concentrations (0-15 mM) of analytes were mixed. The ^1H NMR spectrum was run for each respective concentration of analyte in 10 mM β -CD on a Bruker 300 MHz NMR. ^1H NMR titration experiments were run for analytes **1-4** and fluorophores **6-8**. The NMR spectra were evaluated using MestReNova software. Binding constants were determined using the equation shown below:

$$K_a = \Delta\delta / (\Delta\delta_{\text{tot}} - \Delta\delta[\text{G}])$$

The average K_a value was determined for each analyte and fluorophore.

SUMMARY TABLES FOR BINDING EXPERIMENTS

Table S3. Results of binding constant experiments

Compound number	Average K_a (mM^{-1})
1	0.0667
2	<i>a</i>
3	0.0667
4	0.0167
6	<i>a</i>
7	0.300
8	0.114

^a Indicates nonsensical values for binding constants

SUMMARY TABLE OF FLUORESCENCE MODULATION EXPERIMENTS

Table S4. Results of fluorescence modulation experiments

Analyte	Fluorophore	α -CD	β -CD	Me- β -CD	2-HPCD	PBS
1	6	1.12 $\pm$ 0.00	1.15 $\pm$ 0.01	1.01 $\pm$ 0.01	1.10 $\pm$ 0.01	1.22 $\pm$ 0.02
1	7	1.09 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.02 $\pm$ 0.00	1.00 $\pm$ 0.00
1	8	1.21 $\pm$ 0.01	1.01 $\pm$ 0.03	0.97 $\pm$ 0.01	0.88 $\pm$ 0.01	1.22 $\pm$ 0.01
2	6	1.15 $\pm$ 0.02	1.15 $\pm$ 0.01	0.97 $\pm$ 0.01	1.14 $\pm$ 0.02	1.33 $\pm$ 0.02
2	7	0.98 $\pm$ 0.00	1.00 $\pm$ 0.01	0.99 $\pm$ 0.01	0.97 $\pm$ 0.00	1.00 $\pm$ 0.00
2	8	1.07 $\pm$ 0.02	1.03 $\pm$ 0.04	0.87 $\pm$ 0.02	0.94 $\pm$ 0.01	1.10 $\pm$ 0.00
3	6	1.30 $\pm$ 0.04	1.24 $\pm$ 0.02	0.96 $\pm$ 0.01	1.18 $\pm$ 0.02	1.27 $\pm$ 0.01
3	7	1.08 $\pm$ 0.02	0.96 $\pm$ 0.02	1.04 $\pm$ 0.00	0.99 $\pm$ 0.01	0.88 $\pm$ 0.00
3	8	1.08 $\pm$ 0.03	1.24 $\pm$ 0.14	0.94 $\pm$ 0.01	0.88 $\pm$ 0.00	1.23 $\pm$ 0.01
4	6	1.26 $\pm$ 0.02	1.15 $\pm$ 0.01	0.99 $\pm$ 0.01	1.08 $\pm$ 0.01	1.34 $\pm$ 0.01
4	7	1.28 $\pm$ 0.01	1.05 $\pm$ 0.05	1.03 $\pm$ 0.02	0.99 $\pm$ 0.00	1.09 $\pm$ 0.03
4	8	1.21 $\pm$ 0.01	1.08 $\pm$ 0.13	0.83 $\pm$ 0.02	0.94 $\pm$ 0.00	1.23 $\pm$ 0.00
5	6	1.05 $\pm$ 0.02	1.06 $\pm$ 0.02	0.99 $\pm$ 0.00	1.07 $\pm$ 0.01	1.20 $\pm$ 0.05
5	7	0.98 $\pm$ 0.00	0.98 $\pm$ 0.00	0.98 $\pm$ 0.00	0.99 $\pm$ 0.00	0.98 $\pm$ 0.00
5	8	1.07 $\pm$ 0.01	0.88 $\pm$ 0.01	0.98 $\pm$ 0.00	0.97 $\pm$ 0.01	0.99 $\pm$ 0.01

SUMMARY TABLE OF LIMIT OF DETECTION EXPERIMENTS

Table S5. Results of limit of detection experiments

Analyte	Fluorophore	CD	LOD (μM)	LOQ (μM)	Equation	R ²
1	6	β -CD	82.4	162.3	$y = 126651x + 4E7$	0.9977
1	6	PBS	a	a	$y = -125824x + 3E7$	0.8856
2	6	β -CD	a	a	$y = -137716x + 6E7$	0.9916
2	6	PBS	a	a	$y = 122235x + 2E7$	0.8259
3	6	β -CD	31.3	53.3	$y = 807091x + 4E7$	0.9906
3	6	PBS	a	a	$y = 270685x + 5E7$	0.4862
4	6	β -CD	189	a	$y = -42263x + 7E7$	0.9563
4	6	PBS	5.2	7	$y = 3E6x - 179835$	0.9654

a = nonsensical values were obtained

SUMMARY TABLES OF ARRAY GENERATION EXPERIMENTS

Table S6. Results of array generation for analytes **1-5** with α -cyclodextrin and fluorophores **6-8** as predictors

Jackknifed Classification Matrix

	ACDHeptachlor	ACDLindane	ACDMirex	ACDTHF	ACDcis-chlordane	%correct
ACDHeptachlor	4	0	0	0	0	100
ACDLindane	0	4	0	0	0	100
ACDMirex	0	0	3	0	0	100
ACDTHF	0	0	0	4	0	100
ACDcis-chlordane	0	0	0	0	4	100
Total	4	4	3	4	4	100

Cumulative Proportion of Total Dispersion

0.858	0.997	1.000
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Table S7. Results of array generation for analytes **1-5** with β -cyclodextrin and fluorophores **6-8** as predictors

Jackknifed Classification Matrix

	BCDHeptachlor	BCDLindane	BCDMirex	BCDTHF	BCDcis-chlordane	%correct
BCDHeptachlor	4	0	0	0	0	100
BCDLindane	0	4	0	0	0	100
BCDMirex	0	0	4	0	0	100
BCDTHF	0	0	0	4	0	100
BCDcis-chlordane	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.986	0.999	1.000
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Table S8. Results of array generation for analytes **1-5** with methyl- β -cyclodextrin and fluorophores **6-8** as predictors

Jackknifed Classification Matrix

	MeBCDHeptachlor	MeBCDLindane	MeBCDMirex	MeBCDTHF	MeBCDcis-chlordane	%correct
MeBCDHeptachlor	4	0	0	0	0	100
MeBCDLindane	0	4	0	0	0	100
MeBCDMirex	0	0	4	0	0	100
MeBCDTHF	0	0	0	4	0	100
MeBCDcis-chlordane	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.952	0.982	1.000
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Table S9. Results of array generation for analytes **1-5** with 2-hydroxypropyl- β -cyclodextrin and fluorophores **6-8** as predictors

Jackknifed Classification Matrix

	2HPCDHeptachlor	2HPCDLindane	2HPCDMirex	2HPCDTHF	2HPCDcis-chlordane	%correct
2HPCDHeptachlor	4	0	0	0	0	100
2HPCDLindane	0	4	0	0	0	100
2HPCDMirex	0	0	4	0	0	100
2HPCDTHF	0	0	0	4	0	100
2HPCDcis-chlordane	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.803	0.984	1.000
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Table S10. Results of array generation for analytes **1-5** with phosphate buffered saline and fluorophores **6-8** as predictors

Jackknifed Classification Matrix

	PBSHeptachlor	PBSLindane	PBSMirex	PBSTHF	PBScis-chlordane	%correct
PBSHeptachlor	4	0	0	0	0	100
PBSLindane	0	4	0	0	0	100
PBSMirex	0	0	4	0	0	100
PBSTHF	0	0	0	4	0	100
PBScis-chlordane	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.846	0.999	1.000
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Table S11. Results of array generation for analytes **1-5** with all cyclodextrin hosts and fluorophores **6-8** as predictors

Jackknifed Classification Matrix (Contd.)

	ACDcis-chlordane	BCDHeptachlor	BCDLindane	BCDMirex	BCDTHF	BCDcis-chlordane	MeBCDHeptachlor	MeBCDLindane
2HPCDHeptachlor	0	0	0	0	0	0	0	0
2HPCDLindane	0	0	0	0	0	0	0	0
2HPCDMirex	0	0	0	0	0	0	0	0
2HPCDTHF	0	0	0	0	0	0	0	0
2HPCDcis-chlordane	0	0	0	0	0	0	0	0
ACDHeptachlor	0	0	0	0	0	0	0	0
ACDLindane	0	0	0	0	0	0	0	0
ACDMirex	0	0	0	0	0	0	0	0
ACDTHF	0	0	0	0	0	0	0	0
ACDcis-chlordane	4	0	0	0	0	0	0	0
BCDHeptachlor	0	4	0	0	0	0	0	0
BCDLindane	0	0	4	0	0	0	0	0
BCDMirex	0	0	0	4	0	0	0	0
BCDTHF	0	0	0	0	4	0	0	0
BCDcis-chlordane	0	0	0	0	0	4	0	0
MeBCDHeptachlor	0	0	0	0	0	0	4	0
MeBCDLindane	0	0	0	0	0	0	0	4
MeBCDMirex	0	0	0	0	0	0	0	0
MeBCDTHF	0	0	0	0	0	0	0	0
MeBCDcis-chlordane	0	0	0	0	0	0	0	0
PBSHeptachlor	0	0	0	0	0	0	0	0
PBSLindane	0	0	0	0	0	0	0	0
PBSMirex	0	0	0	0	0	0	0	0
PBSTHF	0	0	0	0	0	0	0	0
PBScis-chlordane	0	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	MeBCDMirex	MeBCDTHF	MeBCDcis-chlordane	PBSHeptachlor	PBSLindane	PBSMirex	PBSTHF	PBScis-chlordane	%correct
2HPCDHeptachlor	0	0	0	0	0	0	0	0	100
2HPCDLindane	0	0	0	0	0	0	0	0	100
2HPCDMirex	0	0	0	0	0	0	0	0	100
2HPCDTHF	0	0	0	0	0	0	0	0	100
2HPCDcis-chlordane	0	0	0	0	0	0	0	0	100
ACDHeptachlor	0	0	0	0	0	0	0	0	100
ACDLindane	0	0	0	0	0	0	0	0	100
ACDMirex	0	0	0	0	0	0	0	0	100
ACDTHF	0	0	0	0	0	0	0	0	100
ACDcis-chlordane	0	0	0	0	0	0	0	0	100
BCDHeptachlor	0	0	0	0	0	0	0	0	100
BCDLindane	0	0	0	0	0	0	0	0	100
BCDMirex	0	0	0	0	0	0	0	0	100
BCDTHF	0	0	0	0	0	0	0	0	100
BCDcis-chlordane	0	0	0	0	0	0	0	0	100
MeBCDHeptachlor	0	0	0	0	0	0	0	0	100
MeBCDLindane	0	0	0	0	0	0	0	0	100
MeBCDMirex	4	0	0	0	0	0	0	0	100
MeBCDTHF	0	4	0	0	0	0	0	0	100
MeBCDcis-chlordane	0	0	4	0	0	0	0	0	100
PBSHeptachlor	0	0	0	4	0	0	0	0	100
PBSLindane	0	0	0	0	4	0	0	0	100
PBSMirex	0	0	0	0	0	4	0	0	100
PBSTHF	0	0	0	0	0	0	4	0	100
PBScis-chlordane	0	0	0	0	0	0	0	4	100
Total	4	4	4	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.714	0.936	1.000
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SUMMARY FIGURES FOR ALL FLUORESCENCE MODULATION EXPERIMENTS

Figure S5. Fluorescence modulation of analyte **1** with fluorophore **6**

Figure S6. Fluorescence modulation of analyte **1** with fluorophore **7**

Figure S7. Fluorescence modulation of analyte **1** with fluorophore **8**

Figure S8. Fluorescence modulation of analyte **2** with fluorophore **6**

Figure S9. Fluorescence modulation of analyte 2 with fluorophore 7

Figure S10. Fluorescence modulation of analyte 2 with fluorophore 8

Figure S11. Fluorescence modulation of analyte **3** with fluorophore **6**

Figure S12. Fluorescence modulation of analyte **3** with fluorophore **7**

Figure S13. Fluorescence modulation of analyte **3** with fluorophore **8**

Figure S14. Fluorescence modulation of analyte **4** with fluorophore **6**

Figure S15. Fluorescence modulation of analyte 4 with fluorophore 7

Figure S16. Fluorescence modulation of analyte 4 with fluorophore 8

Figure S17. Fluorescence modulation of analyte 5 with fluorophore 6

Figure S18. Fluorescence modulation of analyte 5 with fluorophore 7

Figure S19. Fluorescence modulation of analyte **5** with fluorophore **8**

SUMMARY FIGURES FOR LIMIT OF DETECTION EXPERIMENTS

Figure S20. Limit of detection of analyte **1** with fluorophore **6** and β -cyclodextrin

Figure S21. Limit of detection of analyte **1** with fluorophore **6** and phosphate buffered saline

Figure S22. Limit of detection of analyte **2** with fluorophore **6** and β -cyclodextrin

Figure S23. Limit of detection of analyte **2** with fluorophore **6** and phosphate buffered saline

Figure S24. Limit of detection of analyte **3** with fluorophore **6** and β -cyclodextrin

Figure S25. Limit of detection of analyte **3** with fluorophore **6** and phosphate buffered saline

Figure S26. Limit of detection of analyte **4** with fluorophore **6** and β -cyclodextrin

Figure S27. Limit of detection of analyte **4** with fluorophore **6** and phosphate buffered saline

SUMMARY FIGURES FOR ARRAY GENERATION EXPERIMENTS

Figure S28. Linear discriminant analysis of fluorescence responses of analytes **1-5** with α -cyclodextrin with fluorophores **6-8** as predictors

Figure S29. Linear discriminant analysis of fluorescence responses of analytes **1-5** with β -cyclodextrin with fluorophores **6-8** as predictors

Figure S30. Linear discriminant analysis of fluorescence responses of analytes **1-5** with methyl- β -cyclodextrin with fluorophores **6-8** as predictors

Figure S31. Linear discriminant analysis of fluorescence responses of analytes **1-5** with 2-hydroxypropyl- β -cyclodextrin with fluorophores **6-8** as predictors

Figure S32. Linear discriminant analysis of fluorescence responses of analytes **1-5** with phosphate buffered saline with fluorophores **6-8** as predictors

Figure S33. Linear discriminant analysis of fluorescence responses of analytes **1-5** with all cyclodextrin hosts with fluorophores **6-8** as predictors

CHAPTER 3

Published in *ACS Omega* **2017**, 2, 8591-8599

Detection of Organochlorine Pesticides in Contaminated Marine Environments via
Cyclodextrin-Promoted Fluorescence Modulation

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Manuscript 3

Detection of Organochlorine Pesticides in Contaminated Marine Environments via Cyclodextrin-Promoted Fluorescence Modulation

ABSTRACT

The development of practical and robust detection methods for pesticides is an important research objective due to the known toxicity, carcinogenicity, and environmental persistence of these compounds. Pesticides have been found in bodies of water that are located near areas where pesticides are commonly used, and easily spread to beaches, lakes, and rivers, affect the species living in those waterways, and harm humans who come into contact with or eat fish from such water. Reported herein is the rapid, sensitive, and selective detection of four organochlorine pesticides in a variety of water sources across the state of Rhode Island using cyclodextrin-promoted fluorescence detection. This method relies on the ability of cyclodextrin to promote analyte-specific fluorescence modulation of a high quantum yield fluorophore when a pesticide is in close proximity, combined with subsequent array-based statistical analyses of the measurable changes in the emission signals. This system operates with high sensitivity (low micromolar detection limits), selectivity (100% differentiation between structurally similar analytes), and general applicability (for different water samples with varying salinity and pH, as well as for different water temperatures).

INTRODUCTION

Pesticides are a ubiquitous class of organic pollutants known for their environmental persistence,¹⁻³ carcinogenicity,^{4,5} and toxicity to humans,⁶ animals,⁷ and plants.⁸ These compounds are used widely by farmers world-wide in order to control

insects, fungi, and other pests,^{9,10} and to maximize crop yields and food production.^{11,12} Once released into the environment, pesticides spread widely, persisting in the water,¹³ air,¹⁴ soil,¹⁵ and sediment.¹⁶ Many of these pesticides are resistant to hydrolysis and degrade slowly under standard environmental conditions;¹⁷⁻¹⁹ as a result, pesticides both bioaccumulate and biomagnify in the food chain,²⁰ and have exacerbated negative effects to humans and animals.^{21,22}

The ability to detect pesticides is an important research objective with significant applications in the food and agricultural industries,^{23,24} public health policies,^{25,26} and a variety of other scientific sub-disciplines.²⁷ Currently used methods for such detection rely heavily on mass spectrometry-based techniques,²⁸⁻³⁰ which are time-consuming, expensive, and often require additional preparation procedures prior to analysis. Newer detection methods using techniques such as Raman spectroscopy^{31,32} or electrochemical assays³³ have recently been developed and demonstrate extremely high sensitivity; however, each of these techniques also requires significant time and resources, which can limit the ability to conduct high-throughput assays of large populations to monitor widespread pesticide exposure.

Previous work in our laboratory has focused on the use of cyclodextrin-promoted fluorescence energy transfer for the rapid, sensitive, and selective detection of aromatic toxicants.³⁴⁻³⁷ This system relies on the ability of cyclodextrin to promote proximity-induced energy transfer from an aromatic toxicant energy donor to a high quantum yield fluorophore acceptor, leading to a bright, turn-on fluorescence signal, that is unique to each cyclodextrin-analyte-fluorophore combination. We have shown that such detection operates successfully in purified buffer solutions,³⁸ human urine³⁹

and human breast milk,⁴⁰ that it can form part of oil spill remediation procedures using cyclodextrin-promoted toxicant extraction followed by fluorescence detection,⁴¹⁻⁴⁴ and that it can be used for the detection of both polar and non-polar photophysically active toxicants.⁴⁵

In cases where the target analyte is not photophysically active and cannot participate in energy transfer, highly analyte-specific fluorescence modulation can still occur, in which the cyclodextrin promotes proximity-induced changes in the fluorophore emission signal when the target analyte is in close proximity. This fluorescence modulation-based system has been used successfully in the detection of fuel components including benzene and alkylated benzene derivatives in contaminated water samples,⁴⁶ as well as in the detection of non-aromatic alcohols⁴⁷ and organochlorine pesticides⁴⁸ in purified buffer systems.

One environment that is particularly important is water, because toxicants found in aqueous environments will affect people who drink the water, organisms that live in the water (i.e. seafood), and people who consume such organisms. Although our previously-reported cyclodextrin-based systems provided accurate pesticide detection in purified buffer systems, there are substantial differences between such purified buffer solutions and real-world aqueous environments that complicate the development and optimization of toxicant detection methods. In particular, real-world aqueous environments contain a plethora of living species (both plant and animal), can have high salinity (in salt-water systems) and a broad variety of pHs, and can be found at a variety of temperatures (depending on geographic location and season).

Reported herein is the rapid, sensitive, and selective detection of four organochlorine pesticides in water sources across the state of Rhode Island using cyclodextrin-promoted fluorescence modulation. We demonstrate that this system operates with high sensitivity (low micromolar detection limits), selectivity (100% differentiation between compounds), and general applicability (for different water samples with varying salinity and pH, as well as for different temperatures of these water samples).

EXPERIMENTAL

Materials and Methods

All organochlorine pesticides and control analytes (compounds **1-5**, Figure 1) were purchased from Sigma-Aldrich Chemical Company and used as received, unless otherwise noted. Fluorophore **6** was synthesized following literature-reported procedures.⁴⁹ ¹H NMR spectra were obtained using a Bruker 300 MHz spectrometer. UV-Visible spectra were obtained using a Shimadzu UV-3600 Plus spectrophotometer. Fluorescence spectra were obtained using a Shimadzu RF-5301PC spectrophotofluorimeter. The following commercially available cyclodextrin derivatives were used as received: α -cyclodextrin, β -cyclodextrin, randomly methylated β -cyclodextrin,⁵⁰ 2-hydroxypropyl β -cyclodextrin, and γ -cyclodextrin. For the temperature-dependent studies, a Fisher Scientific Isotemp 6200 R20 was used to control the temperature and the spectrophotometer was equipped with a single constant-temperature cell holder. All GCMS measurements were obtained using a Shimadzu GCMS-QP2020 gas chromatograph-mass spectrometer. Conductivity measurements

were obtained using a Thermo Scientific Orion 3-Star Benchtop Conductivity Kit. All pH experiments were performed using a MicroLab FS-522.

Figure 1. Structures of analytes **1-5** and fluorophore **6**

Sample Collection Procedures

Water samples were collected from four locations around Rhode Island (Figure 2): Arcadia Lake, Narragansett Bay, Atlantic Ocean, and Providence River. Samples were collected directly from the water using amber high-density polyethylene (HDPE) bottles obtained from Fisher Scientific, were transported to the University of Rhode Island in a climate-controlled vehicle within one hour of collection, and were stored in the laboratory refrigerator until used for analysis.

Figure 2. Map of sample collection sites. The red marker indicates Arcadia Lake, the green marker shows Narragansett Bay, the blue marker shows the Atlantic Ocean, and the purple marker shows Providence River. The map has been digitally manipulated so that only the state of Rhode Island is in focus.

General Procedure for Fluorescence Modulation Experiments

In a quartz cuvette, 1.25 mL of a 10 mM cyclodextrin solution dissolved in phosphate buffered saline (PBS) and 1.25 mL of the water sample were combined. 100 μ L of a fluorophore **6** solution (0.1 mg/mL in THF) was added, and the solution was excited at 460 nm. 20 μ L of analytes **1-4** (1.0 mg/mL solution in THF) or control analyte **5** were added to the mixture, and the resulting solution was excited at 460 nm. The fluorescence emission spectra were integrated vs. wavenumber on the X-axis, and the fluorescence modulation was measured by the ratio of integrated emission in the

presence of the analyte to integrated emission of the fluorophore in the absence of the analyte, as shown in Equation 1:

$$\text{Fluorescence modulation} = Fl_{\text{analyte}}/Fl_{\text{blank}} \quad (\text{Eq. 1})$$

Where Fl_{analyte} is the integrated fluorescence emission of the fluorophore in the presence of analyte, and Fl_{blank} is the integrated fluorescence emission of the fluorophore in the absence of the analyte. All fluorescence experiments were performed at room temperature (~22 °C)

Temperature-Dependent Fluorescence Modulation Experiments

10 mM solutions of cyclodextrin were prepared in PBS. Fluorescence modulation experiments were conducted at 5 °C and 30 °C, as the vast majority of ocean water temperatures fall into this range.^{51,52} Fluorescence experiments were repeated for each water-cyclodextrin-analyte combination. The temperature control system indicated when the desired temperature was reached, and each sample was allowed to equilibrate for approximately 10 minutes at the final temperature before the fluorescence emission spectrum was collected.

General Procedure for Limit of Detection Calculations⁵³

The limit of detection (LOD) is defined as the lowest concentration of analyte at which a signal can be detected. To determine this value, the following steps were performed for each water-cyclodextrin-analyte combination. In a quartz cuvette, 1.25 mL of 10 mM cyclodextrin in PBS and 1.25 mL of water sample were combined. Then, 100 µL of a 0.1 mg/mL solution of fluorophore **6** was added, the solution was excited at the excitation wavelength of fluorophore **6**, and the fluorescence emission spectra were recorded. Six repeat measurements were taken.

Next, 5 μL of analyte (1 mg/mL in THF) were doped into an aqueous sample, and again the solution was excited at the fluorophore's excitation wavelength, and the fluorescence emission spectra were recorded. Six repeat measurements were taken. This step was repeated for 10 μL of analyte, 15 μL of analyte, 20 μL of analyte, 25 μL of analyte, 30 μL of analyte, 35 μL of analyte, and 40 μL of analyte, all of which were doped into an aqueous sample that did not initially contain toxicants, to measure the ability of the system to detect the doped toxicants within the complex aqueous matrix.

All of the fluorescence emission spectra were integrated vs. wavenumber on the X-axis, and calibration curves were generated. The curves were plotted with the analyte concentration in μM on the X-axis, and the fluorescence modulation ratio on the Y-axis. The curve was fitted to a straight line and the equation of the line was determined (Figure 3).

Figure 3. Example of a calibration curve used to calculate limit of detection

The limit of detection is defined according to Equation 2:

$$\text{LOD} = 3(\text{SD}_{\text{blank}})/m \quad (\text{Eq. 2})$$

Where SD_{blank} is the standard deviation of the blank sample and m is the slope of the calibration curve. In cases where the slope of the trendline was negative, the absolute value of the slope was used to calculate the LOD. In all cases, the LOD was reported in μM .

General Procedure for Array Generation Experiments

Array analysis was performed using SYSTAT 13 statistical computing software with the following settings: (a) Classical Discriminant Analysis; (b) Grouping Variable: Analytes; (c) Predictors: α -cyclodextrin/BODIPY, β -cyclodextrin/BODIPY, methyl- β -cyclodextrin/BODIPY, 2-hydroxypropyl- β -cyclodextrin/BODIPY, γ -cyclodextrin/BODIPY, PBS/BODIPY; (d) Long-range statistics: Mahal

General Procedures for Water Characterization Experiments

GC-MS Experiments

10 mL of the water sample and 2 grams of sodium chloride were added to a separatory funnel. Then, 20 mL of dichloromethane was added to the separatory funnel and the separatory funnel was shaken vigorously. The aqueous layer was discarded, and another 10 mL of water was added to the organic layer. The remaining organic layer was set to evaporate to 1 mL under a stream of nitrogen, filtered, and analyzed by GC-MS.

All GC-MS measurements were obtained on a Shimadzu-QP2020 gas chromatograph-mass spectrometer with the following settings: Column: Shimadzu SH-Rxi-5SilMS (30 m x 0.25 mm x 0.25 μm); Oven Temperature: 45 $^{\circ}\text{C}$, hold for 7 minutes, ramp to 200 $^{\circ}\text{C}$ at 20 $^{\circ}\text{C}/\text{min}$, hold for 60 minutes; Injection Temperature: 200 $^{\circ}\text{C}$; Splitting ratio: Splitless; MS ion source temperature: 230 $^{\circ}\text{C}$; Interface temperature: 150

°C; Total run time: 60 minutes.

pH Experiments

All pH experiments were performed using a Microlab FS-522 equipped with a pH probe. The probe was calibrated using Fisherbrand pH 4, 7, and 10 premade buffer solutions. Data was obtained using Microlab software version 6.3.4.

Conductivity Experiments

All conductivity experiments were performed using a Thermo Scientific Orion 3-Star Benchtop Conductivity Kit. The conductivity probe was rinsed with deionized water between samples.

RESULTS AND DISCUSSION

Water Characterization Experiments

GC-MS Experiments

Quantifiable differences between the locations used for water sampling were determined via GC-MS analysis. All water samples tested showed peaks that corresponded to long-chain alkanes,⁵⁴ fatty acids,⁵⁵ and amides⁵⁶ which are commonly found in marine environments (Figure 4). Notably, Arcadia Lake showed the highest number of peaks, indicating the largest number of organic compounds, and greatest intensity of peaks, indicating the highest concentrations of each of those compounds.

The higher prevalence of organic compounds in Arcadia Lake compared to the other water bodies investigated is likely due to the lack of natural cycling in lake water⁵⁷ compared to the river, ocean, and bay, and the limited options for content regeneration in that lake.⁵⁸ Notably, samples from Arcadia Lake also contained GC-MS peaks that corresponded to phthalates, commonly found in plastics⁵⁹ and other commercial

products,⁶⁰ and benzophenone,⁶¹ commonly used in personal care products such as sunscreen⁶² and lip balm.⁶³

Figure 4. GC-MS overlay of water samples where the black line represents Arcadia Lake, the pink line represents Narragansett Bay, the blue line represents Atlantic Ocean, and the brown line represents Providence River

pH and Conductivity Experiments

Differences between the four water sampling locations were further quantified through pH and conductivity analysis. The pH of water bodies can vary significantly, often becoming more acidic in locations where acid rain is prevalent,⁶⁴ and basic in cases where there are algae blooms⁶⁵ or other ecosystem-wide phenomena.⁶⁶ The most acidic water of the four samples was the Atlantic Ocean, with a measured pH of 6.51.

The most basic water body was Arcadia Lake, with a pH of 8.28, likely as a result of algae growth that is known to be highly prevalent in lake environments. The conductivity of each sample was measured, and used as an indicator of salinity and electrolyte concentration, in accordance with literature precedent.^{67,68} Unsurprisingly, the Atlantic Ocean sample had the highest conductivity, corresponding to the highest salt content, closely followed by the Narragansett Bay (also a salt water location) and the Providence River. Arcadia Lake, a freshwater body, showed a markedly lower conductivity value (lowest salinity), as expected.

Fluorescence Modulation Experiments

Undoped Water Samples

In addition to measuring innate differences in the water samples using GC-MS, pH, and conductivity analyses, we investigated the ability of fluorophore **6** in combination with varying cyclodextrins to report on the differing environments and solution compositions. To that end, analyte-free (termed “undoped”) water samples were mixed with fluorophore **6** and cyclodextrins, and the fluorescence emission signals of fluorophore **6** for each sample-cyclodextrin combination were subjected to linear discriminant analysis (LDA). The results indicate 100% success in differentiating between water samples (Figure 5), which is a result of the sensitivity of fluorophore **6**-cyclodextrin combinations to differences in salinity, pH, and other sample-specific compositional variations.

Figure 5. Array response patterns using linear discriminant analysis of fluorescence response signals of fluorophore **6** in water samples

Analyte-Doped Water Samples

After determining that different aqueous environments translate into different fluorescence emission responses in undoped solutions, we then evaluated the ability of fluorophore **6**-cyclodextrin combinations to detect the presence of toxicants that had been doped into the water, using our previously-developed cyclodextrin-promoted fluorescence modulation. For each water-cyclodextrin-fluorophore **6** combination, we added small amounts of an aliphatic pesticide (compounds **1-4**) as a solution in tetrahydrofuran (THF). We also evaluated the effect of the THF itself without additional pesticide present, as control analyte **5**. The degree of modulated fluorescence emission that occurred with the introduction of analytes **1-4** or control analyte **5** was quantified according to Equation 1. In most cases, the fluorescence modulation values were greater than 1, indicating that introduction of the analytes led to an increase in the fluorescence emission of fluorophore **6** (Table 1).

Table 1. Selected fluorescence modulation results for analytes **1-5** in Atlantic Ocean^a

Analyte	α -CD	β -CD	Me- β -CD	2HPCD	γ -CD	PBS
1	1.16 $\pm$ 0.01	1.06 $\pm$ 0.01	1.03 $\pm$ 0.01	1.06 $\pm$ 0.01	1.05 $\pm$ 0.01	1.15 $\pm$ 0.01
2	1.12 $\pm$ 0.01	1.05 $\pm$ 0.01	1.17 $\pm$ 0.01	1.06 $\pm$ 0.01	1.06 $\pm$ 0.004	1.14 $\pm$ 0.01
3	1.08 $\pm$ 0.02	1.04 $\pm$ 0.004	1.02 $\pm$ 0.004	1.07 $\pm$ 0.01	1.07 $\pm$ 0.01	1.08 $\pm$ 0.001
4	1.13 $\pm$ 0.01	1.05 $\pm$ 0.004	1.04 $\pm$ 0.002	1.08 $\pm$ 0.01	1.07 $\pm$ 0.01	1.11 $\pm$ 0.01
5	1.04 $\pm$ 0.004	1.04 $\pm$ 0.005	1.02 $\pm$ 0.002	1.06 $\pm$ 0.01	1.05 $\pm$ 0.01	1.06 $\pm$ 0.004

^aAll results represent an average of results from four trials for each sample. Fluorescence modulation values were calculated using Equation 1. Errors are shown with enough significant figures to accurately capture the errors.

The different cyclodextrins caused different degrees of changes in the fluorescence emission of fluorophore **6**, which highlights the role of cyclodextrin as a highly specific supramolecular scaffold. Notably, the degree of fluorescence modulation for observed for analytes in the presence of α -cyclodextrin are similar to those observed in the absence of any supramolecular host (PBS), which is likely a result of the fact that α -cyclodextrin is known to have a low affinity for the majority of organic analytes due to its highly sterically constrained cavity.^{69,70} Selected results from Table 1 are highlighted in Figure 6.

Figure 6. Fluorescence changes of fluorophore **6** upon introduction of (A) analyte **1**; (B) analyte **2**; (C) analyte **3**; (D) analyte **4**; and (E) control analyte **5** in the Atlantic Ocean ($\lambda_{\text{ex}} = 460$ nm)

In addition to comparing fluorophore ratios in the presence and absence of analyte, as shown in Table 1, we can also compare fluorescence emission intensities across different cyclodextrin hosts. As seen in Figure 5, the greatest emission intensity for fluorophore **6** was obtained in the presence of methyl- β -cyclodextrin. The high

fluorescence intensity associated with methyl- β -cyclodextrin is well known from our previous research,⁴³⁻⁴⁵ and is likely a result of the high binding affinity of methyl- β -cyclodextrin to fluorophore **6** and related aromatic organic analytes.⁷¹⁻⁷³

Interestingly, the sampling location had an effect on the emission signals as well. The effect of sampling location on analytes **1** and **2** in the presence and absence of cyclodextrin can be seen in Figure 7. The differences between sampling locations are more pronounced and more noticeable with the methyl- β -cyclodextrin host, which highlights advantages in using a host that promotes strong fluorescence emission signals. Differences observed between water sample locations are quantified in Table 2, below.

Figure 7. Fluorescence changes of fluorophore 6 in the various sampling locations in the presence of (A) analyte **1** and Me- β -CD; (B) analyte **1** and PBS; (C) analyte **2** and Me- β -CD; and (D) analyte **2** and PBS

Table 2. Selected fluorescence modulation results comparing sampling location^a

Analyte	Cyclodextrin	Arcadia Lake	Narragansett Bay	Ocean	Providence River
1	Me- β -CD	1.17 ± 0.01	1.26 ± 0.02	1.03 ± 0.01	1.05 ± 0.003
	PBS	1.21 ± 0.02	1.14 ± 0.01	1.15 ± 0.01	1.20 ± 0.01
2	Me- β -CD	1.08 ± 0.01	1.23 ± 0.02	1.17 ± 0.01	1.05 ± 0.01
	PBS	1.28 ± 0.01	1.14 ± 0.01	1.14 ± 0.01	1.18 ± 0.02

^a All results represent an average of results from four trials for each sample. Fluorescence modulation values were calculated using Equation 1. Errors are shown with enough significant figures to accurately capture the errors.

Practical Considerations

The ability to use this cyclodextrin-promoted fluorescence modulation system for the development of practical detection devices requires: (a) **selectivity** in differentiating between structurally similar analytes, (b) **sensitivity** for low concentrations of pesticides, and (c) **general applicability** for different water samples with varying salinity and pH, as well as for different temperatures of these water samples.

Selectivity

The selectivity of this system was determined using array-based analysis to distinguish between structurally similar analytes. Statistical analysis of the response patterns generated from fluorescence-based detection of analytes **1-5** in the presence of several cyclodextrin hosts in each sampling location resulted in 100% differentiation between analytes (Figure 8).

Figure 8. Array-based detection of analytes **1-5** in (A) Arcadia Lake; (B) Narragansett Bay; (C) Atlantic Ocean and (D) Providence River

The response patterns are markedly distinct for each water sample, and show unique, well-separated signals for structurally similar organochlorine pesticides within each water sample. The high degree of success and noticeable visual differences between sampling locations highlight the power of this statistical method in distinguishing even very slight structural variations (*i.e.* analytes **1** and **2**) and in differentiating between sampling locations. Moreover, the results indicate that fluorescence modulation as a basis for toxicant detection operates with high selectivity in complex marine environments, regardless of the salt content (highest in the Atlantic

Ocean), pH (varied between 6.51 and 8.28), or innate toxicant contamination levels (highest in Arcadia Lake).

Additionally, array-based analysis was also performed on a sample from Arcadia Lake to see if this method could differentiate between two structurally-near stereoisomeric compounds, *cis*-chlordanes (analyte **1**) and *trans*-chlordanes (Figure 9).

Figure 9. Array-based detection of analytes **1-5** and *trans*-chlordanes Arcadia Lake

The response patterns show well-separated signals for *cis*-chlordanes and *trans*-chlordanes, even in an area of high innate toxicant contamination levels as in Arcadia Lake. This highlights the power of this method to distinguish between *cis* and *trans* isomers, which is a difficult task even for highly specialized and costly instrumentation.

Sensitivity

The sensitivity of this system was determined by calculating limits of detection for all water-methyl- β -cyclodextrin-analyte combinations following literature-reported procedures, and the results of these studies are summarized in Table 3.

Table 3. Limits of detection for analytes **1-4** in water samples^a

Analyte	Sampling Location	LOD (ppm)
1	Arcadia Lake	1.71 ± 0.02
	Narragansett Bay	0.50 ± 0.002
	Atlantic Ocean	0.41 ± 0.001
	Providence River	2.67 ± 0.01
2	Arcadia Lake	4.25 ± 0.08
	Narragansett Bay	0.81 ± 0.01
	Atlantic Ocean	0.21 ± 0.001
	Providence River	0.55 ± 0.002
3	Arcadia Lake	3.08 ± 0.01
	Narragansett Bay	0.50 ± 0.001
	Atlantic Ocean	0.28 ± 0.002
	Providence River	0.63 ± 0.002
4	Arcadia Lake	2.19 ± 0.02
	Narragansett Bay	5.56 ± 0.03
	Atlantic Ocean	1.07 ± 0.01
	Providence River	1.94 ± 0.004

^aLimits of detection were calculated using the procedures in Cheng 2016; see Electronic Supporting Information for more information. Errors are shown with enough significant figures to accurately capture the errors.

These detection limits are near or below the 0.5 ppm literature-reported levels of concern in many cases.⁷⁴⁻⁷⁶ In general, the limits of detection for analytes in Arcadia Lake were slightly higher than those measured in the other aqueous environments. This is likely a result of the higher innate levels of toxicants found in this lake that can interfere with favorable intermolecular interactions and slightly lower the system sensitivity under those conditions. Current efforts in our laboratory are focused on lowering detection limits for cases where the detection limits are above the literature-reported levels of concern using other fluorophores and/or cyclodextrin hosts. We are

also exploring the use of a pre-concentration step in conjunction with this system to achieve higher effective concentrations of the toxicant analytes.⁷⁷⁻⁷⁹

General Applicability

The general applicability of this system was determined using cyclodextrin-promoted fluorescence modulation and subsequent array-based analyses at varying temperatures, focused particularly on seasonal temperature variations of Rhode Island water to include extreme winter temperatures (5 °C) and extreme summer temperatures (30 °C). Detection at these temperatures resulted in 100% success in differentiating and identifying the pesticide analytes, which highlights the ability of this detection system to operate at a broad variety of temperatures, including most measured ocean temperatures (Figure 10).^{80,81} This selectivity applied even in real-world complicated environments, which contained some amount of innate organic matter, as measured by gas chromatography-mass spectrometry (vide supra) and supported by literature precedent.⁸²

Figure 10. Array-based detection of analytes **1-5** in Arcadia Lake at (A) 5 °C and (B) 30 °C.

Additionally, the general applicability was also highlighted by comparing the fluorescence response signals of a single target analyte in the four different water samples, all of which have differences in their chemical compositions (see GCMS section above). For these experiments, we selected analyte **1**, and the integrated fluorescence responses from fluorophore **6** in the presence of analyte **1** in the four different water samples yielded four unique response signals (Figure 11). Statistical analysis of these results provided 100% success in identifying the water sampling location in which the analyte was found. The well-separated, distinct signals for each water sample highlight the potential for profiling sampling locations based on such identification, and creating water sampling location-specific patterns for accurate identification purposes.

CONCLUSION

In summary, cyclodextrin-promoted fluorescence modulation can be used for the detection of organochlorine pesticides in contaminated marine environments. This method is selective (100% successful in differentiating structurally similar analytes such as *cis*-chlordane and *trans*-chlordane), sensitive (sub-ppm detection limits, with slightly higher limits observed in Arcadia Lake for some analytes), and generally applicable (for different water samples with varying salinity including freshwater and saltwater, and pH, as well as for different temperatures of these water samples including most measured temperatures of ocean water). The high selectivity, sensitivity, and general applicability show potential for the development of practically applicable portable detection devices. Future work in our laboratory will be dedicated towards lowering detection limits to achieve optimal sensitivity and further developing this method into a

portable detection device for the detection of pesticides in contaminated marine environments.

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**Detection of Organochlorine Pesticides in Contaminated Marine Environments
via Cyclodextrin-Promoted Fluorescence Modulation**

MATERIALS AND METHODS

All chemicals were purchased from Sigma-Aldrich Chemical Company and used as received, unless otherwise noted. ^1H NMR spectra were obtained using a Bruker 300 MHz spectrometer. UV-Visible spectra were obtained using a Shimadzu UV-3600 Plus spectrophotometer. Fluorescence spectra were obtained using a Shimadzu RF-5301PC spectrophotofluorimeter. For the temperature-dependent studies, a Fisher Scientific Isotemp 6200 R20 was used to control the temperature and the spectrophotometer was equipped with a single constant-temperature cell holder. All GCMS measurements were obtained using a Shimadzu GCMS-QP2020 gas chromatograph-mass spectrometer. Conductivity measurements were obtained using a Thermo Scientific Orion 3-Star Benchtop Conductivity Kit. All pH experiments were performed using a MicroLab FS-522.

Water samples were collected from four locations around Rhode Island: Arcadia Lake, Narragansett Bay, Atlantic Ocean accessed from the Narragansett Town Beach, and Providence River. Samples were collected directly from the water surface using amber high-density polyethylene (HDPE) bottles obtained from Fisher Scientific and stored in the refrigerator until used.

All spectra were integrated versus wavenumber on the X-axis using OriginPro software. Array analysis was performed using SYSTAT 13 statistical computing software with the following settings:

(a) Classical Discriminant Analysis

(b) Grouping variable: Analytes

(c) Predictors: α -cyclodextrin/BODIPY, β -cyclodextrin/BODIPY, methyl- β -cyclodextrin/BODIPY, 2-hydroxypropyl- β -cyclodextrin, γ -cyclodextrin/BODIPY

(d) Long-range statistics: Mahal

ANALYTE DETAILS

Figure S1. Structures of analytes **1-4**, control analyte **5**, and fluorophore **6**

Table S1. Final solution concentrations of analytes and fluorophores

Compound	Volume (μL)	Final Concentration (μM)
1	20	18.8
2	20	20.6
3	20	26.4
4	20	14.1
5	20	107
6	100	9.5

SYNTHESIS OF FLUOROPHORE 6

The synthesis of BODIPY **6** was performed according to literature-reported procedures: REFERENCE: Shepherd, J. L.; Kell, A.; Chung, E.; Sinclar, C. W.; Workentin, M. S.; Bizzotto, D. *J. Am. Chem. Soc.* **2004**, *126*, 8329-8335.

Reaction 1:

Figure S2. Synthetic pathway to structure **S5**

Procedure: 2.0 grams of 11-bromoundecanoic acid **S1** (7.54 mmol, 1.0 eq.) was combined with 2 drops of *N,N*-dimethylformamide in 40 mL of dichloromethane. 1.0 gram of oxalyl chloride **S2** (7.88 mmol, 1.05 eq.) was dissolved in 5.0 mL of dichloromethane and added dropwise. The reaction mixture was stirred for one hour, then the crude mixture was concentrated on the rotary evaporator and dried on a vacuum overnight to remove any unreacted oxalyl chloride. The resulting acid chloride **S3** was dissolved in 50 mL of dichloromethane. 0.772 mL of 2,4-dimethylpyrrole **S4** (7.50 mmol, 0.99 eq.) was dissolved in 5.0 mL of dichloromethane and added to the reaction mixture. The resulting reaction mixture was heated to reflux for 3 hours under a nitrogen atmosphere, during which time the mixture became a dark red color. After three hours, the reaction mixture was cooled to room temperature and solvent was removed on the rotary evaporator until approximately 5.0 mL of the dichloromethane solution remained. 200 mL of *n*-hexanes were added to the flask, and the mixture was cooled overnight in the freezer at -20 °C. The hexanes were decanted from the insoluble oil and precipitate.

The resulting crude product was dissolved in 75 mL of toluene and heated to 80 oC. 1.0 mL of triethylamine (7.17 mmol, 0.95 eq.) was added and the solution immediately turned light yellow. 1.0 mL of boron trifluoride etherate (8.10 mmol, 1.07 eq.) was then added and the reaction mixture was stirred at 80 oC for 30 minutes, during which time the color of the mixture darkened and became fluorescent. The reaction mixture was cooled to room temperature, and the product was extracted 3 times with brine (50 mL each time). The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography (1:1 dichloromethane: hexanes) to yield the desired product in 28% yield (comparable to the literature-reported 24% yield).

Reaction 2:

Figure S3. Synthetic pathway to structure **S7**

Procedure: Compound **S5** (0.968 g, 2.07 mmol, 1.0 eq.) and compound **S6** (0.27 grams, 2.36 mmol, 1.14 eq.) were dissolved in 50 mL of acetone. The reaction mixture was heated to reflux for two hours. After two hours, the reaction mixture was cooled to room temperature, acetone was removed, and the crude solid was re-dissolved in dichloromethane and washed with water. The organic extract was dried over sodium sulfate, filtered and concentrated, to yield compound **S7** in 97% yield (0.932 grams).

Reaction 3:

Figure S4. Synthetic pathway to compound **6**

Procedure: Compound **S7** (0.932 grams, 2.01 mmol, 1.0 eq.) was dissolved in 150 mL of anhydrous ethanol that was purged with nitrogen. Potassium carbonate was added, and the reaction mixture was warmed to 30 °C. The reaction mixture was stirred under nitrogen for 4 hours at 30 °C. The contents of the flask were poured over 40 mL of aqueous saturated ammonium chloride, at which point the solution turned bright orange. The product was extracted with dichloromethane and washed several times with water. The organic layer was dried over sodium sulfate, filtered, and concentrated. The product was purified via flash chromatography (1:1 dichloromethane: hexanes) to yield compound **6** in 76% yield (674 mg).

¹H NMR OF FLUOROPHORE 6

Figure S5. ¹H NMR spectrum of compound 6

EXPERIMENTAL DETAILS FOR FLUORESCENCE MODULATION EXPERIMENTS

In a quartz cuvette, 1.25 mL of a 10 mM cyclodextrin solution dissolved in phosphate buffered saline (PBS) and 1.25 mL of water sample were combined. 100 μ L of a fluorophore **6** solution (0.1 mg/mL in THF) was added, and the solution was excited at 460 nm, the excitation wavelength used to directly excited fluorophore **6**. 20 μ L of analytes **1-5** (1.0 mg/mL solution in THF) were added to the mixture, and the resulting solution was excited at 460 nm. The fluorescence emission spectra were integrated vs. wavenumber on the X-axis, and fluorescence modulation was measured by the ratio of integrated emission of the fluorophore in the presence of the analyte to integrated emission of the fluorophore in the absence of the analyte, as shown in Equation 1:

$$\text{Fluorescence modulation} = Fl_{\text{analyte}}/Fl_{\text{blank}} \quad (\text{Eq. 1})$$

Where Fl_{analyte} is the integrated fluorescence emission of the fluorophore in the presence of analyte, and Fl_{blank} is the integrated fluorescence emission of the fluorophore in the absence of the analyte.

TEMPERATURE EXPERIMENTS

10 mM cyclodextrin solutions were prepared in phosphate buffered saline (PBS). Fluorescence modulation experiments were then conducted for Arcadia Lake water samples, using the above procedures, at 5 °C and 30 °C. The temperature control system used indicated when the desired temperature was reached, and after the temperature was reached, each sample was allowed to sit in the unit for ~10 minutes before the fluorescence emission spectrum was collected.

EXPERIMENTAL DETAILS FOR LIMIT OF DETECTION EXPERIMENTS

The limit of detection (LOD) is defined as the lowest concentration of analyte at which a signal can be detected. To determine this value, the following steps were performed for each water-cyclodextrin-analyte combination. In a quartz cuvette, 1.25 mL of 10 mM cyclodextrin in PBS and 1.25 mL of water sample were combined. Then, 100 μ L of a 0.1 mg/mL solution of fluorophore **6** was added, the solution was excited at the excitation wavelength of fluorophore **6**, and the fluorescence emission spectra were recorded. Six repeat measurements were taken.

Next, 5 μ L of analyte (1 mg/mL in THF) was added, and again the solution was excited at the fluorophore's excitation wavelength, and the fluorescence emission spectra were recorded. Six repeat measurements were taken. This step was repeated for 10 μ L of analyte, 15 μ L of analyte, 20 μ L of analyte, 25 μ L of analyte, 30 μ L of analyte, 35 μ L of analyte, and 40 μ L of analyte.

All of the fluorescence emission spectra were integrated vs. wavenumber on the X-axis, and calibration curves were generated. The curves plotted the analyte concentration in μ M on the X-axis, and the fluorescence modulation ratio on the Y-axis. The curve was fitted to a straight line and the equation of the line was determined.

The limit of detection is defined according to the following equation:

$$\text{LOD} = 3(\text{SD}_{\text{blank}})/m$$

Where SD_{blank} is the standard deviation of the blank sample and m is the slope of the calibration curve. In cases where the slope of the trend line was negative, the absolute value of the slope was used to calculate the LOD. In all cases, the LOD was calculated in μ M.

EXPERIMENTAL DETAILS FOR ARRAY GENERATION EXPERIMENTS

Array analysis was performed using SYSTAT 13 statistical computing software with the following settings:

(a) Classical Discriminant Analysis

(b) Grouping variable: Analytes

(c) Predictors: α -cyclodextrin/BODIPY, β -cyclodextrin/BODIPY, methyl- β -cyclodextrin/BODIPY, 2-hydroxypropyl- β -cyclodextrin, γ -cyclodextrin/BODIPY

(d) Long-range statistics: Mahal

EXPERIMENTAL DETAILS FOR GC-MS EXPERIMENTS

Extraction Method

These experiments were conducted following literature procedures:

REFERENCE: Park, Y. M.; Pyo, H.; Park, S. J.; Park, S. K. Development of the analytical method for 1,4-dioxane in water by liquid-liquid extraction. *Anal. Chim. Acta* **2005**, 548, 109-115.

10 mL of a water sample and 2 grams of sodium chloride were added to a separatory funnel. Then, 20 mL of dichloromethane was added to the separatory funnel and the separatory funnel was shaken vigorously. The aqueous layer was discarded, and another 10 mL of water was added to the organic layer. The remaining organic layer evaporated to a 1 mL volume under a stream of nitrogen, filtered, and analyzed by GC-MS.

GC-MS Method Details

Column: Shimadzu SH-Rxi-5SilMS (30 m x 0.25 mm x 0.25 μ m); Oven Temperature: 45 $^{\circ}$ C, hold for 7 minutes, ramp to 200 $^{\circ}$ C at 20 $^{\circ}$ C/min, hold for 60 minutes; Injection Temperature: 200 $^{\circ}$ C; Splitting ratio: Splitless; MS ion source temperature: 230 $^{\circ}$ C; Interface temperature: 150 $^{\circ}$ C; Total run time: 60 minutes.

EXPERIMENTAL DETAILS FOR WATER CHARACTERIZATION EXPERIMENTS

pH Experiments

All pH experiments were performed using a Microlab FS-522 equipped with a pH probe. The probe was calibrated using Fisherbrand pH 4, 7, and 10 premade buffer solutions. Data was obtained using Microlab software version 6.3.4.

Conductivity Experiments

All conductivity experiments were performed using a Thermo Scientific Orion 3-Star Benchtop Conductivity Kit. The conductivity probe was rinsed thoroughly with deionized water between samples.

SUMMARY TABLE FOR FLUORESCENCE MODULATION EXPERIMENTS

Table S2. Fluorescence modulation results for each sampling location

Analyte	Water	α -CD	β -CD	Me- β -CD	2HPCD	γ -CD	PBS
1	Arcadia Lake	1.21 $\pm$ 0.01	1.06 $\pm$ 0.00	1.17 $\pm$ 0.01	1.03 $\pm$ 0.00	1.08 $\pm$ 0.02	1.21 $\pm$ 0.02
2		1.24 $\pm$ 0.02	1.14 $\pm$ 0.01	1.08 $\pm$ 0.01	1.34 $\pm$ 0.03	1.16 $\pm$ 0.01	1.28 $\pm$ 0.01
3		1.09 $\pm$ 0.00	1.08 $\pm$ 0.00	1.02 $\pm$ 0.01	1.04 $\pm$ 0.01	1.07 $\pm$ 0.01	1.07 $\pm$ 0.00
4		1.11 $\pm$ 0.01	1.06 $\pm$ 0.01	1.01 $\pm$ 0.01	1.04 $\pm$ 0.01	1.02 $\pm$ 0.00	1.10 $\pm$ 0.01
5		1.04 $\pm$ 0.01	1.04 $\pm$ 0.01	1.01 $\pm$ 0.01	1.02 $\pm$ 0.01	1.02 $\pm$ 0.01	1.07 $\pm$ 0.01
1	Narragansett Bay	1.13 $\pm$ 0.01	1.07 $\pm$ 0.01	1.26 $\pm$ 0.02	1.09 $\pm$ 0.01	1.08 $\pm$ 0.01	1.14 $\pm$ 0.01
2		1.15 $\pm$ 0.01	1.05 $\pm$ 0.00	1.23 $\pm$ 0.02	1.06 $\pm$ 0.01	1.05 $\pm$ 0.00	1.14 $\pm$ 0.01
3		1.06 $\pm$ 0.00	1.07 $\pm$ 0.00	1.18 $\pm$ 0.02	1.06 $\pm$ 0.01	1.08 $\pm$ 0.01	1.08 $\pm$ 0.01
4		1.10 $\pm$ 0.01	1.04 $\pm$ 0.01	1.02 $\pm$ 0.01	1.05 $\pm$ 0.00	1.03 $\pm$ 0.00	1.08 $\pm$ 0.01
5		1.06 $\pm$ 0.02	1.05 $\pm$ 0.01	1.01 $\pm$ 0.00	1.06 $\pm$ 0.00	1.03 $\pm$ 0.01	1.06 $\pm$ 0.01
1	Atlantic Ocean	1.16 $\pm$ 0.01	1.06 $\pm$ 0.01	1.03 $\pm$ 0.01	1.06 $\pm$ 0.01	1.05 $\pm$ 0.01	1.15 $\pm$ 0.01
2		1.12 $\pm$ 0.01	1.05 $\pm$ 0.01	1.17 $\pm$ 0.01	1.06 $\pm$ 0.01	1.06 $\pm$ 0.00	1.14 $\pm$ 0.01
3		1.08 $\pm$ 0.02	1.04 $\pm$ 0.00	1.02 $\pm$ 0.00	1.07 $\pm$ 0.01	1.07 $\pm$ 0.01	1.08 $\pm$ 0.00
4		1.13 $\pm$ 0.01	1.05 $\pm$ 0.00	1.04 $\pm$ 0.00	1.08 $\pm$ 0.01	1.07 $\pm$ 0.01	1.11 $\pm$ 0.01
5		1.04 $\pm$ 0.00	1.04 $\pm$ 0.00	1.02 $\pm$ 0.00	1.06 $\pm$ 0.01	1.05 $\pm$ 0.01	1.06 $\pm$ 0.00
1	Providence River	1.20 $\pm$ 0.02	1.06 $\pm$ 0.01	1.05 $\pm$ 0.00	1.06 $\pm$ 0.01	1.07 $\pm$ 0.00	1.20 $\pm$ 0.01
2		1.16 $\pm$ 0.02	1.05 $\pm$ 0.01	1.05 $\pm$ 0.00	1.08 $\pm$ 0.01	1.05 $\pm$ 0.00	1.18 $\pm$ 0.02
3		1.09 $\pm$ 0.01	1.10 $\pm$ 0.01	1.08 $\pm$ 0.01	1.04 $\pm$ 0.00	1.06 $\pm$ 0.00	1.16 $\pm$ 0.02
4		1.12 $\pm$ 0.03	1.07 $\pm$ 0.00	1.02 $\pm$ 0.00	1.05 $\pm$ 0.00	1.04 $\pm$ 0.00	1.10 $\pm$ 0.01
5		1.05 $\pm$ 0.01	1.03 $\pm$ 0.00	1.02 $\pm$ 0.01	1.03 $\pm$ 0.00	1.02 $\pm$ 0.01	1.04 $\pm$ 0.00

SUMMARY TABLES FOR ARRAY GENERATION EXPERIMENTS

Table S3. Results of array generation for Arcadia Lake

Jackknifed Classification Matrix

	Chlordane	Heptachlor	Lindane	Mirex	THF	%correct
Chlordane	4	0	0	0	0	100
Heptachlor	0	4	0	0	0	100
Lindane	0	0	4	0	0	100
Mirex	0	0	0	4	0	100
THF	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.694	0.870	0.966	1.000
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Table S4. Results of array generation for Narragansett Bay

Jackknifed Classification Matrix

	Chlordane	Heptachlor	Lindane	Mirex	THF	%correct
Chlordane	4	0	0	0	0	100
Heptachlor	0	4	0	0	0	100
Lindane	0	0	4	0	0	100
Mirex	0	0	0	4	0	100
THF	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.706	0.867	0.973	1.000
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Table S5. Results of array generation for Atlantic Ocean

Jackknifed Classification Matrix

	Chlordane	Heptachlor	Lindane	Mirex	THF	%correct
Chlordane	4	0	0	0	0	100
Heptachlor	0	4	0	0	0	100
Lindane	0	0	4	0	0	100
Mirex	0	0	0	4	0	100
THF	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.673	0.914	0.983	1.000
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Table S6. Results of array generation for Providence River

Jackknifed Classification Matrix

	Chlordane	Heptachlor	Lindane	Mirex	THF	%correct
Chlordane	4	0	0	0	0	100
Heptachlor	0	4	0	0	0	100
Lindane	0	0	4	0	0	100
Mirex	0	0	0	4	0	100
THF	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.817	0.940	0.994	1.000
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SUMMARY TABLE FOR LIMIT OF DETECTION EXPERIMENTS

Table S7. Limits of detection for analytes with fluorophore 6

Analyte	Water	Equation	R ²	LOD (μM)
1	Arcadia Lake	$y = 0.0075x + 1.0153$	0.99460	4.18
2		$y = 0.0022x + 1.0032$	0.99861	11.4
3		$y = 0.0015x + 1.0019$	0.99965	10.6
4		$y = 0.0038x + 1.002$	0.99938	4.02
1	Narragansett Bay	$y = 0.0117x + 1.006$	0.99625	1.21
2		$y = 0.0077x + 1.0722$	0.99299	2.16
3		$y = 0.0061x + 0.9977$	0.99262	1.73
4		$y = -0.00x + 0.9975$	0.99696	10.2
1	Atlantic Ocean	$y = 0.0034x + 0.9642$	0.99719	1.00
2		$y = 0.0072x + 0.9626$	0.99477	0.574
3		$y = 0.003x + 0.98080$	0.99250	0.944
4		$y = 0.0035x + 1.0035$	0.99766	1.97
1	Providence River	$y = 0.0021x + 1.0085$	0.99792	6.51
2		$y = 0.0035x + 0.9861$	0.99634	1.47
3		$y = 0.0029x + 0.9974$	0.99755	2.18
4		$y = 0.0047x + 1.0074$	0.99972	3.56

SUMMARY TABLE FOR TEMPERATURE EXPERIMENTS

Table S8. Fluorescence modulation results at 5 °C and 30 °C

Analyte	Water	Temperature (°C)	α -CD	β -CD	Me- β -CD	2HPCD	γ -CD	PBS
1	Arcadia Lake	5	1.09 ± 0.00	1.08 ± 0.01	1.01 ± 0.01	1.06 ± 0.00	1.06 ± 0.02	1.09 ± 0.01
		30	1.13 ± 0.01	1.10 ± 0.01	1.05 ± 0.00	1.14 ± 0.01	1.11 ± 0.01	1.21 ± 0.04
2	Arcadia Lake	5	1.11 ± 0.01	1.08 ± 0.01	1.07 ± 0.00	1.12 ± 0.01	1.05 ± 0.00	1.09 ± 0.01
		30	1.07 ± 0.01	1.03 ± 0.01	1.01 ± 0.00	1.04 ± 0.01	1.04 ± 0.00	1.09 ± 0.01
3	Arcadia Lake	5	1.06 ± 0.00	1.08 ± 0.00	1.04 ± 0.00	1.07 ± 0.00	1.06 ± 0.01	1.09 ± 0.01
		30	1.06 ± 0.01	1.05 ± 0.01	1.00 ± 0.00	1.04 ± 0.00	1.03 ± 0.00	1.07 ± 0.00
4	Arcadia Lake	5	1.11 ± 0.01	1.08 ± 0.00	1.03 ± 0.00	1.05 ± 0.00	1.05 ± 0.00	1.08 ± 0.01
		30	1.06 ± 0.00	1.06 ± 0.00	1.00 ± 0.00	1.05 ± 0.00	1.06 ± 0.00	1.09 ± 0.02
5	Arcadia Lake	5	1.05 ± 0.01	1.04 ± 0.01	1.01 ± 0.00	1.01 ± 0.01	1.03 ± 0.02	1.09 ± 0.01
		30	1.05 ± 0.01	1.05 ± 0.01	1.00 ± 0.00	1.04 ± 0.00	1.05 ± 0.00	1.08 ± 0.01

SUMMARY TABLES FOR TEMPERATURE ARRAYS

Table S9. Results of array generation for Arcadia Lake at 5 °C

Jackknifed Classification Matrix

	Chlordane	Heptachlor	Lindane	Mirex	THF	%correct
Chlordane	4	0	0	0	0	100
Heptachlor	0	4	0	0	0	100
Lindane	0	0	4	0	0	100
Mirex	0	0	0	4	0	100
THF	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.644	0.835	0.947	1.000
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Table S10. Results of array generation for Arcadia Lake at 30 °C

Jackknifed Classification Matrix

	Chlordane	Heptachlor	Lindane	Mirex	THF	%correct
Chlordane	4	0	0	0	0	100
Heptachlor	0	4	0	0	0	100
Lindane	0	0	4	0	0	100
Mirex	0	0	0	4	0	100
THF	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.704	0.902	0.994	1.000
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SUMMARY TABLES FOR GC-MS EXPERIMENTS

Table S11. GC-MS results for Arcadia Lake

Sample Type	Retention Time (min)	NIST Compound ID
Doped Standard	17.31	Lindane
	19.28	Heptachlor
	24.60	Chlordane
	54.22	Mirex
Undoped Sample	9.36	2,4,4-Trimethyl-1-hexene
	11.45	Nonanal
	12.31	Dodecane
	12.67	Benzothiazole
	13.35	Epoxy-linalooloxide
	13.67	3-Hydroxy-2,2,4-trimethylpentyl 2-methylpropanoate
	13.82	Tetradecane
	14.26	2,5-cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-hydroxy-4-methyl-
	14.32	Cyclododecane
	14.55	3,5-Di-tert-butylphenol
	14.66	2-Heptenoic acid, isobutyl ester
	14.70	Eicosane
	14.78	2-methyloctacosane
	14.81	9-Methylnonadecane
	15.08	Diethyl phthalate
	15.13	Heptadecane
	15.29	Isopropyl laurate
	15.50	Benzophenone
	15.69	Oleic acid amide
	15.80	Pentadecyl acrylate
	16.15	Azunol
	16.36	2-(2,4,4-trimethylpentan-2-yl)phenol
	16.48	4-Formyl-2,6-di-tert-butylphenol
	16.63	8-Octadecanone
	16.70	Carbonic acid, but-3-yn-1-yl tetradecyl ester
	16.77	Tetracosane
	16.84	Tritetracontane
	16.90	4-Methyl-2-benzylphenol
	17.73	n-Nonadecanol-1
	17.88	Octatriacontyl pentafluoropropionate
	18.14	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
	18.61	Benzyl 1,2-dihydroxyethyl carbonate
	18.85	Phthalic acid, 5-methylhex-2-yl butyl ester
	19.07	Lauramide
	19.64	Tetracosane
	19.97	2-Phenyltetradecane

Table S12. GC-MS results for Narragansett Bay

Sample Type	Retention Time (min)	NIST Compound ID
Doped Standard	17.24	Lindane
	19.22	Heptachlor
	24.52	Chlordane
	54.16	Mirex
Undoped Sample	9.67	(1,2,2-Trimethylbutyl)cyclohexane
	9.74	2,4,4-Trimethyl-1-hexene
	10.63	1,4-Dimethylbicyclo[2.1.0]pentane
	10.82	Sulfurous acid, hexyl undecyl ester
	10.99	2-Chlorocyclohexanol
	11.17	Cyclooctyl acetate
	11.54	1,2-Dichlorocyclohexane
	11.86	3-Acetyl-2,5-dimethylfuran
	12.18	trans-Traumatic acid
	12.72	5,6-Dimethyldecane
	12.84	5-Sec-butylnonane
	13.15	4-Hydroxyheptanohydrazide

Table S13. GC-MS results for Atlantic Ocean

Sample Type	Retention Time (min)	NIST Compound ID
Doped Standard	17.33	Lindane
	19.30	Heptachlor
	24.65	Chlordane
	54.33	Mirex
Undoped Sample	9.17	1,2,3,4-Pentamethylcyclopentane
	9.68	2,4,4-Trimethyl-1-hexene
	9.72	3-Ethyl-2-methyl-1-heptene
	13.56	Glycerol, 1, 2-diacetate
	14.12	Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester
	14.24	Tetradecane
	14.46	Benzene, (1,4-cyclohexadien-1-yl)-
	15.57	Heptadecane
	15.78	Dodecanoic acid, 1-methylethyl ester
	16.08	Benzophenone
	16.38	2-Propenoic acid, tridecyl ester
	16.9	5,15-Dimethylnonadecane
	17.47	Tetracosane

Table S14. GC-MS results for Providence River

Sample Type	Retention Time (min)	NIST Compound ID
Doped Standard	17.31	Lindane
	19.29	Heptachlor
	24.61	Chlordane
	54.29	Mirex
Undoped Samples	11.86	n-Undecanal
	14.97	2,4-Di-tert-butylphenol
	15.15	Oleic acid amide
	15.50	10-Octadecenoamide
	16.32	Ethyl tetradecyl ether
	17.84	Stearamide
	18.36	Adipic acid
	19.07	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
	20.15	Dodecanamide
	25.43	Crodamide
	26.27	Hexadecanamide

SUMMARY TABLES FOR WATER CHARACTERIZATION EXPERIMENTS

pH Experiments

Table S15. Results of pH experiments for each sampling location

Water	pH
Arcadia Lake	8.28
Narragansett Bay	7.21
Atlantic Ocean	6.51
Providence River	7.22

Conductivity Experiments

Table S16. Results of conductivity experiments for each sampling location

Water	Conductivity ($\mu\text{S/cm}$)
Arcadia Lake	141.1
Narragansett Bay	31100
Atlantic Ocean	31500
Providence River	2067

SUMMARY FIGURES FOR FLUORESCENCE MODULATION EXPERIMENTS

The black line represents the emission from the fluorophore, and the red line represents the emission from the analyte and fluorophore mixed together in the complex water environment. All X-axes measure the emission from 470 nm to 800 nm, and all Y-axes have been normalized so that the fluorescence emission is on a scale of 0.0 to 1.0.

Figure S6. Fluorescence modulation of analyte **1** in Arcadia Lake

Figure S7. Fluorescence modulation of analyte 2 in Arcadia Lake

Figure S8. Fluorescence modulation of analyte 3 in Arcadia Lake

Figure S9. Fluorescence modulation of analyte 4 in Arcadia Lake

Figure S10. Fluorescence modulation of analyte 5 in Arcadia Lake

Figure S11. Fluorescence modulation of analyte **1** in Narragansett Bay

Figure S12. Fluorescence modulation of analyte **2** in Narragansett Bay

Figure S13. Fluorescence modulation of analyte 3 in Narragansett Bay

Figure S14. Fluorescence modulation of analyte 4 in Narragansett Bay

Figure S15. Fluorescence modulation of analyte 5 in Narragansett Bay

Figure S16. Fluorescence modulation of analyte 1 in Atlantic Ocean

Figure S17. Fluorescence modulation of analyte 2 in Atlantic Ocean

Figure S18. Fluorescence modulation of analyte 3 in Atlantic Ocean

Figure S19. Fluorescence modulation of analyte 4 in Atlantic Ocean

Figure S20. Fluorescence modulation of analyte 5 in Atlantic Ocean

Figure S21. Fluorescence modulation of analyte 1 in Providence River

Figure S22. Fluorescence modulation of analyte 2 in Providence River

Figure S23. Fluorescence modulation of analyte **3** in Providence River

Figure S24. Fluorescence modulation of analyte **4** in Providence River

Figure S25. Fluorescence modulation of analyte **5** in Providence River

SUMMARY FIGURES FOR ARRAY GENERATION

Figure S26. Linear discriminant analysis of fluorescence responses for analytes **1-5** with fluorophore **6** in Arcadia Lake

Figure S27. Linear discriminant analysis of fluorescence responses for analytes **1-5** with fluorophore **6** in Narragansett Bay

Figure S28. Linear discriminant analysis of fluorescence responses for analytes **1-5** with fluorophore **6** in Atlantic Ocean

Figure S29. Linear discriminant analysis of fluorescence responses for analytes **1-5** with fluorophore **6** in Providence River

SUMMARY FIGURES FOR LIMIT OF DETECTION (LOD) EXPERIMENTS

Limits of detection were calculated following literature-reported procedures (vide supra). We plotted the ratio of $Fl_{\text{analyte}}/Fl_{\text{blank}}$ on the Y-axis, and the analyte concentration in micromolar on the X-axis.

Figure S30. Limit of detection for analyte **1** in Arcadia Lake

Figure S31. Limit of detection for analyte **2** in Arcadia Lake

Figure S32. Limit of detection for analyte **3** in Arcadia Lake

Figure S33. Limit of detection for analyte **4** in Arcadia Lake

Figure S34. Limit of detection for analyte **1** in Narragansett Bay

Figure S35. Limit of detection for analyte **2** in Narragansett Bay

Figure S36. Limit of detection for analyte **3** in Narragansett Bay

Figure S37. Limit of detection for analyte **4** in Narragansett Bay

Figure S38. Limit of detection for analyte **1** in Atlantic Ocean

Figure S39. Limit of detection for analyte **2** in Atlantic Ocean

Figure S40. Limit of detection for analyte **3** in Atlantic Ocean

Figure S41. Limit of detection for analyte **4** in Atlantic Ocean

Figure S42. Limit of detection for analyte **1** in Providence River

Figure S43. Limit of detection for analyte **2** in Providence River

Figure S44. Limit of detection for analyte **3** in Providence River

Figure S45. Limit of detection for analyte **4** in Providence River

SUMMARY FIGURES FOR TEMPERATURE EXPERIMENTS

The black line represents fluorophore excitation and the red line represents mixtures of analyte and fluorophore. The X-axis measures emission from 470 nm to 800 nm, and the Y-axis measures the normalized fluorescence emission spectrum from 0.0 to 1.0.

Figure S46. Fluorescence modulation results at 5 °C and 30 °C for analyte 1-α-cyclodextrin-Arcadia Lake

Figure S47. Fluorescence modulation results at 5 °C and 30 °C for analyte 1-β-cyclodextrin-Arcadia Lake

Figure S48. Fluorescence modulation results at 5 °C and 30 °C for analyte 1-methyl- β -cyclodextrin-Arcadia Lake

Figure S49. Fluorescence modulation results at 5 °C and 30 °C for analyte 1-2-hydroxypropyl- β -cyclodextrin-Arcadia Lake

Figure S50. Fluorescence modulation results at 5 °C and 30 °C for analyte 1- γ -cyclodextrin-Arcadia Lake

Figure S51. Fluorescence modulation results at 5 °C and 30 °C for analyte 1-phosphate buffered saline-Arcadia Lake

Figure S52. Fluorescence modulation results at 5 °C and 30 °C for analyte 2- α -cyclodextrin-Arcadia Lake

Figure S53. Fluorescence modulation results at 5 °C and 30 °C for analyte 2- β -cyclodextrin-Arcadia Lake

Figure S54. Fluorescence modulation results at 5 °C and 30 °C for analyte 2-methyl- β -cyclodextrin-Arcadia Lake

Figure S55. Fluorescence modulation results at 5 °C and 30 °C for analyte 2-2-hydroxypropyl- β -cyclodextrin-Arcadia Lake

Figure S56. Fluorescence modulation results at 5 °C and 30 °C for analyte 2- γ -cyclodextrin-Arcadia Lake

Figure S57. Fluorescence modulation results at 5 °C and 30 °C for analyte 2-phosphate buffered saline-Arcadia Lake

Figure S58. Fluorescence modulation results at 5 °C and 30 °C for analyte 3- α -cyclodextrin-Arcadia Lake

Figure S59. Fluorescence modulation results at 5 °C and 30 °C for analyte 3- β -cyclodextrin-Arcadia Lake

Figure S60. Fluorescence modulation results at 5 °C and 30 °C for analyte **3**-methyl- β -cyclodextrin-Arcadia Lake

Figure S61. Fluorescence modulation results at 5 °C and 30 °C for analyte **3**-2-hydroxypropyl- β -cyclodextrin-Arcadia Lake

Figure S62. Fluorescence modulation results at 5 °C and 30 °C for analyte **3**- γ -cyclodextrin-Arcadia Lake

Figure S63. Fluorescence modulation results at 5 °C and 30 °C for analyte 3-phosphate buffered saline-Arcadia Lake

Figure S64. Fluorescence modulation results at 5 °C and 30 °C for analyte 4- α -cyclodextrin-Arcadia Lake

Figure S65. Fluorescence modulation results at 5 °C and 30 °C for analyte 4- β -cyclodextrin-Arcadia Lake

Figure S66. Fluorescence modulation results at 5 °C and 30 °C for analyte 4-methyl- β -cyclodextrin-Arcadia Lake

Figure S67. Fluorescence modulation results at 5 °C and 30 °C for analyte 4-2-hydroxypropyl- β -cyclodextrin-Arcadia Lake

Figure S68. Fluorescence modulation results at 5 °C and 30 °C for analyte 4- γ -cyclodextrin-Arcadia Lake

Figure S69. Fluorescence modulation results at 5 °C and 30 °C for analyte 4-phosphate buffered saline-Arcadia Lake

Figure S70. Fluorescence modulation results at 5 °C and 30 °C for analyte 5-α-cyclodextrin-Arcadia Lake

Figure S71. Fluorescence modulation results at 5 °C and 30 °C for analyte 5-β-cyclodextrin-Arcadia Lake

Figure S72. Fluorescence modulation results at 5 °C and 30 °C for analyte **5**-methyl-β-cyclodextrin-Arcadia Lake

Figure S73. Fluorescence modulation results at 5 °C and 30 °C for analyte **5**-2-hydroxypropyl-β-cyclodextrin-Arcadia Lake

Figure S74. Fluorescence modulation results at 5 °C and 30 °C for analyte **5**-γ-cyclodextrin-Arcadia Lake

Figure S75. Fluorescence modulation results at 5 °C and 30 °C for analyte **5**-phosphate buffered saline-Arcadia Lake

SUMMARY FIGURES FOR TEMPERATURE ARRAYS

Figure S76. Linear discriminant analysis of fluorescence responses of analytes **1-5** with fluorophore **6** at 5 °C in Arcadia Lake

Figure S77. Linear discriminant analysis of fluorescence responses of analytes **1-5** with fluorophore **6** at 30 °C in Arcadia Lake

SUMMARY FIGURES FOR GC-MS EXPERIMENTS

Figure S78. GC-MS trace for Arcadia Lake lindane-doped standard at 17.31 minutes

Figure S79. GC-MS trace for Arcadia Lake heptachlor-doped standard at 19.28 minutes

Figure S80. GC-MS trace for Arcadia Lake chlordane-doped standard at 24.60 minutes

Figure S81. GC-MS trace for Arcadia Lake mirex-doped standard at 54.22 minutes

Figure S82. GC-MS trace for Arcadia Lake for undoped sample at 9.36 minutes

Figure S83. GC-MS trace for Arcadia Lake for undoped sample at 11.45 minutes

Figure S84. GC-MS trace for Arcadia Lake for undoped sample at 12.31 minutes

Figure S85. GC-MS trace for Arcadia Lake for undoped sample at 12.67 minutes

Figure S86. GC-MS trace for Arcadia Lake for undoped sample at 13.35 minutes

Figure S87. GC-MS trace for Arcadia Lake for undoped sample at 13.67 minutes

Figure S88. GC-MS trace for Arcadia Lake for undoped sample at 13.82 minutes

Figure S89. GC-MS trace for Arcadia Lake for undoped sample at 14.26 minutes

Figure S90. GC-MS trace for Arcadia Lake for undoped sample at 14.32 minutes

Figure S91. GC-MS trace for Arcadia Lake for undoped sample at 14.55 minutes

Figure S92. GC-MS trace for Arcadia Lake for undoped sample at 14.66 minutes

Figure S93. GC-MS trace for Arcadia Lake for undoped sample at 14.70 minutes

Figure S94. GC-MS trace for Arcadia Lake for undoped sample at 14.78 minutes

Figure S95. GC-MS trace for Arcadia Lake for undoped sample at 14.81 minutes

Figure S96. GC-MS trace for Arcadia Lake for undoped sample at 15.08 minutes

Figure S97. GC-MS trace for Arcadia Lake for undoped sample at 15.13 minutes

Figure S98. GC-MS trace for Arcadia Lake for undoped sample at 15.29 minutes

Figure S99. GC-MS trace for Arcadia Lake for undoped sample at 15.50 minutes

Figure S100. GC-MS trace for Arcadia Lake for undoped sample at 15.69 minutes

Figure S101. GC-MS trace for Arcadia Lake for undoped sample at 15.80 minutes

Figure S102. GC-MS trace for Arcadia Lake for undoped sample at 16.15 minutes

Figure S103. GC-MS trace for Arcadia Lake for undoped sample at 16.36 minutes

Figure S104. GC-MS trace for Arcadia Lake for undoped sample at 16.48 minutes

Figure S105. GC-MS trace for Arcadia Lake for undoped sample at 16.63 minutes

Figure S106. GC-MS trace for Arcadia Lake for undoped sample at 16.70 minutes

Figure S107. GC-MS trace for Arcadia Lake for undoped sample at 16.77 minutes

Figure S108. GC-MS trace for Arcadia Lake for undoped sample at 16.84 minutes

Figure S109. GC-MS trace for Arcadia Lake for undoped sample at 16.90 minutes

Figure S110. GC-MS trace for Arcadia Lake for undoped sample at 17.73 minutes

Figure S111. GC-MS trace for Arcadia Lake for undoped sample at 17.88 minutes

Figure S112. GC-MS trace for Arcadia Lake for undoped sample at 18.14 minutes

Figure S113. GC-MS trace for Arcadia Lake for undoped sample at 18.61 minutes

Figure S114. GC-MS trace for Arcadia Lake for undoped sample at 18.85 minutes

Figure S115. GC-MS trace for Arcadia Lake for undoped sample at 19.07 minutes

Figure S116. GC-MS trace for Arcadia Lake for undoped sample at 19.64 minutes

Figure S117. GC-MS trace for Arcadia Lake for undoped sample at 19.97 minutes

Figure S118. GC-MS trace for Narragansett Bay lindane-doped standard at 17.24 minutes

Figure S119. GC-MS trace for Narragansett Bay heptachlor-doped standard at 19.22 minutes

Figure S120. GC-MS trace for Narragansett Bay chlordane-doped standard at 24.52 minutes

Figure S121. GC-MS trace for Narragansett Bay mirex-doped standard at 54.16 minutes

Figure S122. GC-MS trace for Narragansett Bay undoped sample at 9.67 minutes

Figure S123. GC-MS trace for Narragansett Bay undoped sample at 9.74 minutes

Figure S124. GC-MS trace for Narragansett Bay undoped sample at 10.63 minutes

Figure S125. GC-MS trace for Narragansett Bay undoped sample at 10.82 minutes

Figure S126. GC-MS trace for Narragansett Bay undoped sample at 10.99 minutes

Figure S127. GC-MS trace for Narragansett Bay undoped sample at 11.17 minutes

Figure S128. GC-MS trace for Narragansett Bay undoped sample at 11.54 minutes

Figure S129. GC-MS trace for Narragansett Bay undoped sample at 11.86 minutes

Figure S130. GC-MS trace for Narragansett Bay undoped sample at 12.18 minutes

Figure S131. GC-MS trace for Narragansett Bay undoped sample at 12.72 minutes

Figure S132. GC-MS trace for Narragansett Bay undoped sample at 12.84 minutes

Figure S133. GC-MS trace for Narragansett Bay undoped sample at 13.15 minutes

Figure S134. GC-MS trace for Atlantic Ocean lindane-doped standard at 17.33 minutes

Figure S135. GC-MS trace for Atlantic Ocean heptachlor-doped standard at 19.30 minutes

Figure S136. GC-MS trace for Atlantic Ocean chlordane-doped standard at 24.65 minutes

Figure S137. GC-MS trace for Atlantic Ocean mirex-doped standard at 54.33 minutes

Figure S138. GC-MS trace for Atlantic Ocean undoped sample at 9.17 minutes

Figure S139. GC-MS trace for Atlantic Ocean undoped sample at 9.68 minutes

Figure S140. GC-MS trace for Atlantic Ocean undoped sample at 9.72 minutes

Figure S141. GC-MS trace for Atlantic Ocean undoped sample at 13.56 minutes

Figure S142. GC-MS trace for Atlantic Ocean undoped sample at 14.12 minutes

Figure S143. GC-MS trace for Atlantic Ocean undoped sample at 14.24 minutes

Figure S144. GC-MS trace for Atlantic Ocean undoped sample at 14.46 minutes

Figure S145. GC-MS trace for Atlantic Ocean undoped sample at 15.57 minutes

Figure S146. GC-MS trace for Atlantic Ocean undoped sample at 15.78 minutes

Figure S147. GC-MS trace for Atlantic Ocean undoped sample at 16.08 minutes

Figure S148. GC-MS trace for Atlantic Ocean undoped sample at 16.38 minutes

Figure S149. GC-MS trace for Atlantic Ocean undoped sample at 16.90 minutes

Figure S150. GC-MS trace for Atlantic Ocean undoped sample at 17.47 minutes

Figure S151. GC-MS trace for Providence River lindane-doped standard at 17.31 minutes

Figure S152. GC-MS trace for Providence River heptachlor-doped standard at 19.29 minutes

Figure S154. GC-MS trace for Providence River mirex-doped standard at 54.29 minutes

Figure S155. GC-MS trace for Providence River undoped sample at 11.86 minutes

Figure S156. GC-MS trace for Providence River undoped sample at 14.97 minutes

Figure S157. GC-MS trace for Providence River undoped sample at 15.15 minutes

Figure S158. GC-MS trace for Providence River undoped sample at 15.50 minutes

Figure S159. GC-MS trace for Providence River undoped sample at 16.32 minutes

Figure S160. GC-MS trace for Providence River undoped sample at 17.84 minutes

Figure S161. GC-MS trace for Providence River undoped sample at 18.36 minutes

Figure S162. GC-MS trace for Providence River undoped sample at 19.07 minutes

Figure S163. GC-MS trace for Providence River undoped sample at 20.15 minutes

Figure S164. GC-MS trace for Providence River undoped sample at 25.43 minutes

Figure S165. GC-MS trace for Providence River undoped sample at 26.27 minutes

CHAPTER 4

Published in *CLEAN- Soil, Air, Water* **2016**, *44*, 1621-1627

Detection of Benzene and Alkylated Benzene Derivatives in Fuel Contaminated Environments

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Detection of Benzene and Alkylated Benzene Derivatives in Fuel Contaminated Environments

ABSTRACT

Reported herein is the sensitive and selective detection of benzene and alkylated benzene derivatives as well as the practical application of this system in the detection of fuel contaminated environments following a March 2015 tanker truck crash at the Bourne Rotary in Bourne, Massachusetts. This detection method relies on the ability of cyclodextrin to promote proximity-induced fluorescence modulation of a high quantum yield fluorophore, which led to unique modulation responses for each cyclodextrin-analyte-fluorophore combination investigated. The measured changes in fluorescence emission were used to generate arrays using linear discriminant analysis to generate unique pattern identifiers for each combination. The method was successful in detecting ppm levels of analytes with 100% success in differentiating analytes in purified buffer solution and 100% success in differentiating between analytes in water samples from three collection locations related to the crash site: downstream, upstream, and directly from the drainage pipe. Notably, this method was successful in generating well-separated signals for structural isomers of xylene (*ortho*, *meta*, *para*) which is difficult without specialized mass spectrometry-based techniques. The high selectivity, sensitivity, and broad applicability of this method have significant potential in the detection of aromatic analytes in a wide range of complex environments.

INTRODUCTION

The detection of benzene and alkylated benzene derivatives remains a high

priority due to these compounds' known toxicity and carcinogenicity,¹⁻⁴ as well as to their prevalence in a variety of fuels and fuel additives.^{5,6} The high vapor pressures associated with many of these compounds^{7,8} means that in the event of an anthropogenic spill or contamination event, individuals' exposure to such toxicants via airborne routes is likely to be facile and widespread.^{9,10} This is precisely what occurred in March 2015 when a tanker truck crashed at the Bourne rotary in Bourne, Massachusetts, spilling over 5000 gallons of fuel. The extent of the contamination included air (from the vapors of the aromatic analytes), soil (from the fuel that soaked into the soil), and water (from fuel that drained directly into the Cape Cod canal).

There are some options that exist for the environmental remediation of fuel spills;¹¹⁻¹⁵ however, the high cost of many of these options combined with the degree of technical skill necessary to execute such options safely and efficiently prevents many first responders from using such options in the event of the spill. In many cases, the first responders do little to no active cleanup and merely wait for the volatile compounds to evaporate.^{16,17} In the case of the Bourne rotary spill, the first responders monitored the extent of the contamination and removed visible fuel-soaked soil from the scene, but no further direct cleanup efforts were initiated. There is a pressing need for the rapid, sensitive, and selective detection of benzene and alkylated benzene derivatives in fuel contaminated environments without relying on third party reporters. The ability to monitor the extent of this contamination and rationally guide remediation efforts accordingly would represent a significant advance and directly enable more efficient disaster response efforts.

Our group has previously developed the use of cyclodextrin-promoted energy

transfer as a tool for the sensitive and selective detection of toxicants in complex environments.¹⁸⁻²² Such systems have also been used for tandem extraction followed by fluorescence detection in oil spill remediation efforts,²³⁻²⁶ as well as for the deplanarization of aromatic compounds using cyclodextrin-promoted Diels Alder reactions of polycyclic aromatic hydrocarbons.²⁷

Figure 1. Schematic illustration of cyclodextrin-promoted fluorescence modulation

Even in cases where the target analyte does not participate in fluorescence energy transfer, cyclodextrin-promoted fluorescence modulation can still detect such analytes, which include aliphatic organochlorine pesticides and aliphatic alcohols (Fig. 1).^{28,29}

Benzene and alkylbenzene derivatives, including toluene and xylenes (*ortho*, *meta*, and *para* isomers), are unlikely to participate in energy transfer to high quantum yield fluorophores due to their limited photophysical activity and negligible spectral overlap; nonetheless, they are still capable of participating in cyclodextrin-promoted fluorescence modulation. Reported herein is the use of cyclodextrin-promoted fluorescence modulation for precisely this objective: the sensitive and selective detection of benzene and alkylbenzene derivatives, as well as the practical application of this system in the detection of aromatic fuel contaminants from the Bourne, MA fuel spill site.

Materials and Methods

Chemical structures of monomers 1-6 are shown. Monomers 1-5 are aromatic hydrocarbons: 1 (benzene), 2 (toluene), 3 (m-xylene), 4 (o-xylene), and 5 (p-xylene). Monomer 6 is a complex aromatic boron compound featuring a central boron atom bonded to two fluorine atoms and two nitrogen atoms, which are part of a fused ring system with methyl substituents.

Details for Fluorescence Modulation Experiments

$$\text{Fluorophore Ratio} = Fl_{\text{analyte}}/Fl_{\text{blank}} \quad (1)$$

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the analyte, and Fl_{blank} is the fluorescence emission of the fluorophore in the absence of the analyte. Each experiment was repeated four times, and the reported values are the average of the results.

Details for Array Generation Experiments

Array analysis was performed using SYSTAT 13 statistical computing software with the following settings: (a) Classical discriminant analysis; (b) Grouping variable: analytes; (c) Predictors: Bodipy, Rhodamine 6G, Coumarin 6; (d) Long-range statistics: Mahal

Details for Limit of Detection Experiments

These experiments were conducted following literature-reported procedures to determine the minimum amount of analyte necessary to generate a noticeable signal (limit of detection) as well as the amount necessary to generate a quantifiable signal (limit of quantification).³¹

Details of Sample Collection

Glass jars were thoroughly cleaned and dried in an oven prior to use to ensure they were free from contamination. The glass jars were given to first responders at the site on the day of the spill as part of the sample collection methods (not as part of site cleanup). Three samples were collected, each at approximately one foot below the surface of the water: one sample at the pipe that was draining fuel into the Cape Cod canal, the second sample from approximately 100 m upstream from the pipe that was draining fuel into the canal, and the third sample approximately 100 m downstream from the pipe that was draining fuel into the canal. The jars were closed and sealed with tape, and the samples were stored in a refrigerator until use.

RESULTS AND DISCUSSION

Laboratory Experimental Results

In purified buffer solution (phosphate-buffered saline buffered (PBS) at pH 7.4), the introduction of compounds **1**–**5** to a solution of fluorophore **6** in cyclodextrin led to notable changes in the emission of compound **6** (Table 1, Fig. 2), with the degree of change quantified using Eq. (1), above. In most cases the fluorophore ratios were <1, indicating that introduction of the analyte led to a decrease in the fluorescence emission of the fluorophore. Methyl- β -cyclodextrin was the only host that led to increases in the fluorescence emission of compound **6** with the introduction of all aromatic analytes, which is likely related to the known propensity of methyl- β -cyclodextrin to form strong association complexes with fluorophore **6** and promote strong fluorescence emission.³²

The introduction of structural isomers **3**–**5** led to different fluorescence modulation patterns when combined with cyclodextrin hosts and fluorophore **6** (Table 1).

Table 1. Fluorescence modulation of fluorophore **6** in cyclodextrin solutions with the introduction of small amounts of aromatic analytes **1**–**5**^a

Analyte	α -CD	β -CD	Me- β -CD	2-HPCD	PBS
1	0.96 $\pm$ 0.01	0.92 $\pm$ 0.01	1.03 $\pm$ 0.01	0.99 $\pm$ 0.02	0.87 $\pm$ 0.02
2	0.88 $\pm$ 0.01	0.85 $\pm$ 0.01	1.05 $\pm$ 0.00	0.98 $\pm$ 0.01	0.95 $\pm$ 0.00
3	1.00 $\pm$ 0.01	0.97 $\pm$ 0.01	1.06 $\pm$ 0.00	0.98 $\pm$ 0.01	0.92 $\pm$ 0.00
4	0.94 $\pm$ 0.01	0.94 $\pm$ 0.00	1.06 $\pm$ 0.00	0.96 $\pm$ 0.01	0.92 $\pm$ 0.02
5	0.95 $\pm$ 0.01	0.91 $\pm$ 0.02	1.08 $\pm$ 0.00	0.99 $\pm$ 0.01	0.92 $\pm$ 0.01

α -CD, α -cyclodextrin; β -CD, β -cyclodextrin; Me- β -CD, methyl- β -cyclodextrin; 2HPCD, 2-hydroxypropyl- β -cyclodextrin.

^a Fluorescence modulation results calculated using Eq. (1). All results represent the average of four trials.

Figure 2. Modulated fluorescence intensity of fluorophore **6** in the presence of (A) analyte **1**; (B) analyte **2**, and (C) analyte **3**. Black line = fluorescence emission in the absence of any cyclodextrin; red line = fluorescence emission in the presence of α -cyclodextrin; blue line = fluorescence emission in the presence of β -cyclodextrin; purple line = fluorescence emission in the presence of methyl- β -cyclodextrin; and green line = fluorescence emission in the presence of 2-hydroxypropyl- β -cyclodextrin

That differentiation, in turn, directly enabled the generation of an array using linear discriminant analysis that separated the fluorescence response patterns of analytes **1-5** with 100% successful differentiation (Fig. 3). This is particularly noteworthy for structural isomers **3-5**, which are notoriously difficult to differentiate using standard mass spectral based techniques, although more advanced mass spectral based methods have been developed in recent years.^{33,34} Analogous array-based detection systems have been used by a number of research groups for a wide variety of applications, including environmental detection,³⁵ medical diagnostics,³⁶ and explosive detection,³⁷ and the excellent separation of isomers **3-5** highlights the power of this statistical method.

Limits of detection were calculated for all analytes with all cyclodextrin hosts, and selected results of these experiments are summarized in Table 2. The permissible exposure limit for compound **1** is extremely low (1 ppm) and the calculated limit of detection for this compound is close to the permissible exposure limit. However, the other analytes have permissible exposure limits that are several orders of magnitude higher, which means that this newly developed fluorescence modulation-based

detection of analytes **2--5** can detect these aromatic toxicants at concentrations well below the permissible exposure limits. Such sensitive (low limits of detection) and selective (differentiation for individual xylene isomers) detection has significant potential in the development of practical detection devices.

Table 2. Selected limits of detection for analytes **1-5** in fluorophore **6** cyclodextrin solutions^a

Analyte	Host	LOD (ppm)	Permissible exposure limit
1	β -CD	1.13	1 ppm [38]
2	β -CD	0.212	100 ppm [39]
3	β -CD	0.594	100 ppm [40]
4	β -CD	1.20	100 ppm [40]
5	β -CD	1.21	100 ppm [40]

β -CD, beta-cyclodextrin.

^a All results calculated using literature-reported procedures; see Supporting Information for more details.

Figure 3. Array-based separation of analytes **1-5** using fluorescence modulation of fluorophore **6** in various cyclodextrin solutions

Contaminated Fuel Environment

This fluorescence modulation detection system was then applied to water samples taken from a pipe draining into the Cape Cod canal in the immediate aftermath of the spill. For comparison, aqueous samples were also collected upstream from where the pipe drained into the canal (approximately 100 m away) and downstream from where the pipe drained into the canal (100 m away). Characterization of the aqueous samples using fluorescence spectroscopy indicates that the water collected directly from the pipe contained markedly higher levels of photophysically active compounds (Fig. 4), as measured by excitation of the water samples at 250, 360, and 460 nm, and that the water collected downstream from the pipe contained slightly more photophysically active compounds than the control water collected upstream. These results are further confirmed by GC-MS of the aqueous samples, which indicates the samples collected directly from the pipe showed higher abundances of organic compounds as compared to downstream and upstream sample locations (see Supporting Information for more details).

Figure 4. Comparison of the fluorescence response of aqueous samples collected from a pipe eluent, upstream of the eluent, and downstream of the eluent from (A) 250 nm excitation; (B) 360 nm excitation; and (C) 460 nm excitation.

Linear discriminant analysis of the compounds found in these complex

environmental samples was performed by adding fluorophore **6** and a variety of cyclodextrin hosts to each sample, with the cyclodextrin dissolved in either phosphate buffered saline (Fig. 5A) or in ultrapure water (Fig. 5B). The variability in the fluorophore emission signal in the different environments and with the different cyclodextrin hosts was measured, and the results show that samples collected from the three different sites generate well-separated signals and that the pattern of the responses differs depending on whether the cyclodextrin was dissolved in ultrapure water or in phosphate buffered saline. This solvent dependence of cyclodextrin-promoted host-guest phenomena is something that we²³ and others^{41,42} have noted previously.

Figure 5. Linear discriminant analysis of the fluorescence response patterns in undoped complex environments using cyclodextrins dissolved in (A) phosphate-buffered saline and (B) ultrapure water.

Fluorophore comparison ratios were obtained in these complex aqueous samples by comparing the fluorescence emission of fluorophore **6** (dissolved in a 1:1 mixture of the complex aqueous solution and cyclodextrin in PBS) in the absence of an added analyte to its emission in the presence of doped amounts of analytes **1--5**, and some selected results of these experiments are highlighted in Table 3.

Table 3. Fluorescence modulation of fluorophore **6** in complex samples with added cyclodextrin and with the introduction of aromatic analytes **1-5**^a

Analyte	CD	Downstream	Pipe	Upstream
1	α -CD	1.13 $\pm$ 0.01	1.13 $\pm$ 0.02	1.12 $\pm$ 0.01
2	α -CD	1.17 $\pm$ 0.00	1.26 $\pm$ 0.01	1.15 $\pm$ 0.01
3	α -CD	1.32 $\pm$ 0.01	1.29 $\pm$ 0.01	1.40 $\pm$ 0.01
4	α -CD	1.18 $\pm$ 0.00	1.26 $\pm$ 0.02	1.14 $\pm$ 0.01
5	α -CD	1.11 $\pm$ 0.03	1.16 $\pm$ 0.01	1.14 $\pm$ 0.02
1	Me- β -CD	0.98 $\pm$ 0.00	0.97 $\pm$ 0.01	0.98 $\pm$ 0.01
2	Me- β -CD	0.94 $\pm$ 0.00	0.94 $\pm$ 0.01	0.96 $\pm$ 0.00
3	Me- β -CD	0.96 $\pm$ 0.01	0.96 $\pm$ 0.02	0.95 $\pm$ 0.00
4	Me- β -CD	0.96 $\pm$ 0.01	0.96 $\pm$ 0.02	0.95 $\pm$ 0.00
5	Me- β -CD	0.97 $\pm$ 0.01	1.00 $\pm$ 0.02	0.94 $\pm$ 0.02
1	2-HPCD	1.04 $\pm$ 0.00	1.06 $\pm$ 0.04	1.05 $\pm$ 0.01
2	2-HPCD	1.04 $\pm$ 0.01	1.11 $\pm$ 0.00	1.04 $\pm$ 0.01
3	2-HPCD	1.11 $\pm$ 0.00	1.55 $\pm$ 0.02	1.17 $\pm$ 0.01
4	2-HPCD	1.07 $\pm$ 0.01	1.12 $\pm$ 0.01	1.05 $\pm$ 0.00
5	2-HPCD	1.05 $\pm$ 0.01	1.09 $\pm$ 0.00	1.05 $\pm$ 0.01

α -CD, alpha-cyclodextrin; β -CD, beta-cyclodextrin; Me- β -CD, methyl-beta-cyclodextrin; 2HPCD, 2-hydroxypropyl-beta-cyclodextrin.

^a Fluorescence modulation results calculated using Equation 1. All results represent the average of 4 trials.

In most cases, samples collected directly from the pipe showed the highest fluorescence modulation ratios; however, methyl- β -cyclodextrin showed little variability based on sample collection site. Essentially the same results were seen in the downstream, pipe, and upstream samples for a particular analyte-cyclodextrin situation. This is likely due to the tendency of methyl- β -cyclodextrin to form strong association complexes with fluorophores in general that variability and environmental changes such as location have little to no effect on these strongly bound association complexes.³²

Previous work in our group has demonstrated this high performance of methyl- β -cyclodextrin as a host in cyclodextrin-promoted energy transfer and fluorescence modulation due to its hydrophobic character which promotes strong binding of hydrophobic guests such as fluorophore **6**.²³ The greatest degree of variability was seen

for 2-HPCD, which is known to be a good host for extracting compounds from complex oil environments, as we have found previously.²⁵ Other groups have also found that 2-HPCD is particularly efficient at extracting aromatic compounds.^{43,44} Figure 6 highlights a particular example where samples containing 2-HPCD show notable spectral differences depending on the collection site. Similarly, Fig. 7 highlights how the degree of fluorescence modulation in benzene-containing samples is strongly dependent on the identity of the cyclodextrin as well as the sample collection location. The sensitive and selective detection of benzene remains an ongoing concern due to its known toxicity and high carcinogenicity.^{45,46}

Figure 6. Illustration of fluorescence modulation in 2-HPCD-containing samples of analyte **3** collected from (a) downstream from the fuel spill; (B) at the fuel spill pipe; (C) and upstream from the fuel spill.

Figure 7. Illustration of the detection of analyte **1** using cyclodextrin hosts in samples collected from (A) downstream from the eluent pipe; (B) at the site of the eluent pipe; and (C) upstream from the eluent pipe.

Selectivity using array-based detection of these complex aqueous samples was also accomplished using linear discriminant analysis, and some key results are highlighted in Figure 8. Notably, unique patterns are seen based on the location of sample collection. This is true despite the fact that many of the cyclodextrin-analyte combinations showed only small variability in Table 3; nonetheless, even such slight variability is sufficient enough to enable visually distinct array-based response patterns. This highlights the utility of the LDA methodology for obtaining selectivity in detection systems such as those reported herein.

Figure 8. Array-based detection of analytes **1-5** using fluorescence modulation in samples collected from (A) downstream of the spill; (B) at the spill pipe; and (C) upstream of the spill.

CONCLUSIONS

Cyclodextrins can enable proximity-induced interactions between a high quantum yield fluorophore and small molecule analyte, wherein the small molecule analyte influences the fluorescence emission behavior of the fluorophore in a manner specific to each analyte--fluorophore--cyclodextrin combination. These unique signals were used to successfully generate an array that separated 100% of the analytes in both purified buffer solutions and in complex, fuel-contaminated environmental samples. The fact that the emission signals are unique to each analyte-fluorophore combination provides

a distinct advantage in analyte detection: even structurally similar analytes such as *m*-xylene, *o*-xylene, and *p*-xylene lead to well-separated, unique pattern identifiers. The high selectivity, sensitivity, and broad applicability of this system have significant potential in the development of practical detection systems for aromatic analytes in complex environments. Efforts towards the development of such practical systems are currently underway in our laboratory, and results of these and other investigations will be reported in due course.

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**Detection of Benzene and Alkylated Benzene Derivatives in Fuel Contaminated
Environments**

ABBREVIATIONS

Tetrahydrofuran-THF

Cyclodextrin- CD

Alpha-cyclodextrin- α -CD

Beta-cyclodextrin- β -CD

Methyl-beta-cyclodextrin- Me- β -CD

2-hydroxypropyl-beta-cyclodextrin- 2HPCD

Phosphate Buffered Saline- PBS

Linear Discriminant Analysis- LDA

Limit of Detection- LOD

Limit of Quantification – LOQ

MATERIALS AND METHODS

All chemicals were purchased from Sigma-Aldrich Chemical Company and used as received, unless otherwise noted. ^1H -NMR spectra were obtained using a Bruker 300 MHz spectrometer. UV-vis spectra were obtained using an Agilent 8453 spectrometer equipped with a photodiode array detector. Fluorescence spectra were obtained using a Shimadzu RF-5301PC spectrophotofluorimeter.

All spectra were integrated versus wavenumber on the X-axis using OriginPro software. Array analysis was performed using SYSTAT 13 statistical computing software with the following settings:

- (a) Classical discriminant analysis
- (b) Grouping variables: analytes
- (c) Predictors: α -cyclodextrin, β -cyclodextrin, methyl- β -cyclodextrin, 2-hydroxypropyl- β -cyclodextrin, γ -cyclodextrin, and phosphate buffered saline.
- (d) Long-range statistics: Mahal

Water samples from each collection location (downstream, pipe, upstream) were prepared for GC-MS through extraction using water sample/methylene chloride, 1:1 v/v. The organic solvent was dried with sodium sulfate and evaporated using the rotary evaporator. A portion of each sample was used for GC-MS, and the spectra were obtained on an Agilent 6890 Gas Chromatograph-Agilent 5973 Mass Spectrometer.

SYNTHESIS OF FLUOROPHORE **6**

The synthesis of BODIPY **6** was performed according to literature procedures:

REFERENCE: J. L. Shepherd, A. Kell, E. Chung, C. W. Sinclair, M. S. Workentin, D. Bizzotto, *J. Am. Chem. Soc.* **2004**, *126*, 8329.

Reaction 1:

Figure S1. Synthetic pathway to structure **S5**

Procedure: 2 g of 11-bromoundecanoic acid **S1** (7.54 mmol, 1 eq.) was combined with two drops of *N,N*-dimethylformamide in 40 mL of dichloromethane. 1 g of oxalyl chloride **S2** (7.88 mmol, 1.05 eq.) was dissolved in 5 mL of dichloromethane and added dropwise. The reaction mixture was stirred for 1 h, then the crude mixture was concentrated on the rotary evaporator and dried on a vacuum overnight to remove any unreacted oxalyl chloride. The resulting acid chloride **S3** was dissolved in 50 mL of dichloromethane. 0.772 mL of 2,4-dimethylpyrrole **S4** (7.50 mmol, 0.99 eq.) was dissolved in 5 mL of dichloromethane and added to the reaction mixture. The resulting reaction mixture was heated to reflux for 3 h under a nitrogen atmosphere, during which time the mixture became a dark red color. After 3 h, the reaction mixture was cooled to room temperature and the solvent was removed on the rotary evaporator until approximately 5 mL of the dichloromethane solution remained. 200 mL of *n*-hexanes were added to the flask, and the mixture was cooled overnight in the freezer at -20°C .

The hexanes were decanted from the insoluble oil and precipitate. The resulting crude product was dissolved in 75 mL of toluene and heated to 80 °C. 1 mL of triethylamine (7.17 mmol, 0.95 eq.) was added and the solution immediately turned light yellow. 1 mL of boron trifluoride etherate (8.10 mmol, 1.07 eq.) was then added and the reaction mixture was stirred at 80 °C for 30 min, during which time the color of the mixture darkened and became fluorescent. The reaction mixture was cooled to room temperature, and the product was extracted three times with brine (50 mL each time). The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography (dichloromethane/hexanes, 1:1) to yield the desired product in 28% yield (comparable to the literature-reported 24% yield).

Reaction 2:

Figure S2. Synthetic pathway to structure **S7**

Procedure: Compound **S5** (0.968 g, 2.07 mmol, 1 eq.) and compound **S6** (0.27 g, 2.36 mmol, 1.14 eq.) were dissolved in 50 mL of acetone. The reaction mixture was heated to reflux for 2 h. After 2 h, the reaction mixture was cooled to room temperature, acetone was removed, and the crude solid was re-dissolved in dichloromethane and washed with water. The organic extract was dried over sodium sulfate, filtered and concentrated, to yield compound **S7** in 97% yield (0.932 g).

Reaction 3:

Figure S3. Synthetic pathway to compound **6**

Procedure: Compound **S7** (0.932 g, 2.01 mmol, 1 eq.) was dissolved in 150 mL of anhydrous ethanol that was purged with nitrogen. Potassium carbonate was added, and the reaction mixture was warmed to 30 °C. The reaction mixture was stirred under nitrogen for 4 h at 30 °C. The contents of the flask were poured over 40 mL of aqueous saturated ammonium chloride, at which point the solution turned bright orange. The product was extracted with dichloromethane and washed several times with water. The organic layer was dried over sodium sulfate, filtered, and concentrated. The product was purified via flash chromatography (dichloromethane/hexanes, 1:1) to yield compound **6** in 76% yield (674 mg).

¹H NMR OF FLUOROPHORE 6

Figure S4. ¹H NMR of fluorophore 6

ANALYTE DETAILS

Figure S5. Structures of analytes **1-5** and fluorophore **6**

All analytes were prepared at a concentration of 1 mg/mL in tetrahydrofuran (THF).

Fluorophore **6** was prepared at 0.1 mg/mL in THF. The final concentration of all analytes and fluorophore **6** are shown in the table below.

Table S1. Final analyte and fluorophore concentrations

Analyte	Amount added (μL)	Final concentration (μM)
1	20	98.5
2	20	83.5
3	20	72.5
4	20	72.5
5	20	72.5
Fluorophore 6	100	9.5

FLUORESCENCE MODULATION EXPERIMENTS

In a quartz cuvette, 2.5 mL of a 10 mM aqueous cyclodextrin solution in phosphate-buffered saline (PBS) and 100 μL of a fluorophore **6** solution (0.1 mg/mL in THF) were combined. The solution was excited at 460 nm. 20 μL of a 1 mg/mL analyte solution in THF was added to the cuvette, and fluorescence measurements were repeated using 460 nm excitation. The fluorescence spectra were integrated vs. wavenumber on the X -axis, and fluorescence modulation was determined by quantifying a fluorophore ratio according to Eq. (1):

$$\text{Fluorophore ratio} = F_{\text{alcohol}}/F_{\text{blank}} \quad (1)$$

Where F_{alcohol} is the fluorescence emission of the fluorophore in the presence of the alcohol, and F_{blank} is the fluorescence emission of the fluorophore in the absence of the alcohol. Each experiment was repeated four times. The reported values are the average of the results.

ARRAY GENERATION EXPERIMENTAL DETAILS

Array analysis was performed using SYSTAT 13 statistical computing software with the following settings: Classical discriminant analysis; Grouping variable: analytes; Predictors: α -cyclodextrin, β -cyclodextrin, methyl- β -cyclodextrin, 2-hydroxypropyl- β -cyclodextrin, γ -cyclodextrin, and phosphate buffered saline; Long-range statistics: Mahal

Separate arrays were generated for each sample location.

GAS CHROMATOGRAPHY EXPERIMENTS

A water sample from each collection location (downstream, pipe, upstream) were prepared for GC-MS analysis through extraction using water sample/methylene chloride, 1:1 v/v. The organic solvent was dried with sodium sulfate and evaporated on the rotary evaporator. A portion of each sample was used for GC-MS analysis, and the spectra were obtained on an Agilent 6890 GC and Agilent 5973 MS spectrometer.

EXPERIMENTAL DETAILS FOR LIMIT OF DETECTION EXPERIMENTS

The limit of detection (LOD) is defined as the lowest concentration of analyte at which a signal can be detected. The limit of quantification is defined at the lowest concentration of analyte that can be accurately quantified. These experiments were conducted following literature-reported procedures.

REFERENCE: Saute, B.; Premasiri, R.; Ziegler, L.; Narayanan, R. “Gold Nanorods as Surface Enhanced Raman Spectroscopy Substrates for Sensitive and Selective Detection of Ultra-Low Levels of Dithiocarbamate Pesticides.” *Analyst* **2012**, *137*, 5082-5087.

To determine LOD and limit of quantification (LOQ), each fluorophore-analyte-cyclodextrin combination was examined in the following manner:

1. 2.5 mL of 10 mM cyclodextrin in phosphate-buffered saline (PBS) was measured into a cuvette and 100 μ L of a fluorophore solution (0.1 mg/mL) in THF was added. The solution was excited at the fluorophore’s excitation wavelength and the fluorescence emission spectrum was recorded. Six repeat measurements were made for the fluorescence emission spectra.
2. 20 μ L of the analyte was added to the cuvette and the solution was again excited at the fluorophore’s excitation wavelength. Six repeat measurements were taken.
3. Step 2 was repeated for 40 μ L of analyte, 60 μ L of analyte, 80 μ L of analyte, and 100 μ L of analyte. In each case, the solution was excited at the fluorophore’s excitation wavelength and the fluorescence emission spectrum was recorded four times.
4. All fluorescence emission spectra were integrated vs. wavenumber, and calibration curves were generated with the analyte concentration on the *X*-axis (in μ M) and the integrated fluorophore emission on the *Y*-axis. The curve was then fitted to a straight line and an equation for the line was determined.
5. For each case, the fluorophore with cyclodextrin (before any analyte was added) was also excited at its excitation wavelength, and the fluorescence emission spectrum was recorded (as per step 1). These measurements are referred to as the “blank.”
6. The limit of the blank is defined according to the following equation:

$$\text{LoBLOD} = m_{\text{blank}} + 3 (\text{SD}_{\text{blank}}) \quad (2)$$

where m is the mean of the blank integrations and SD is the standard deviation.

7. The limit of the blank was then entered into the equation determined in step 4 (for the y -value), and the corresponding X -value was determined. This value provided the LOD in μM .

8. LOQ was calculated in a similar way to the limit of detection. First, the limit of the blank for quantification was determined according to the following equation:

$$\text{LoBLOQ} = m_{\text{blank}} + 10 (\text{SD}_{\text{blank}}) \quad (3)$$

This value was entered into the equation determined in step 4 (for the y -value), and the corresponding X -value was determined to be the limit of quantification in μM .

GC-MS OF SAMPLE LOCATIONS

Downstream collection site:

Figure S6. Full GC spectrum of downstream collection site

Figure S7. Mass spectrum at 5 min and match with the *o*-xylene reference spectrum

Figure S8. Mass spectrum at 4.35 min and match with the *p*-xylene reference spectrum

Pipe collection site:

Figure S9. Full GC spectrum of pipe collection site

Figure S10. Mass spectrum at 4.35 min and match with the *p*-xylene reference spectrum

Figure S11. Mass spectrum at 5 min and match with the *o*-xylene reference spectrum

Upstream collection site:

Figure S12. Full GC spectrum of upstream collection site

Figure S13. Mass spectrum at 5 min and match with the *o*-xylene reference spectrum

Figure S14. Mass spectrum at 4.35 min and match with the *p*-xylene reference spectrum

SUMMARY TABLES FOR FLUORESCENCE MODULATION

Table S2. Fluorescence modulation results of fluorophore **6** with analytes **1-5** in purified buffer solution

analyte	α -CD	β -CD	Me- β -CD	2-HPCD	PBS
<i>p</i> -xylene	0.95 ± 0.01	0.91 ± 0.02	1.08 ± 0.00	0.99 ± 0.01	0.92 ± 0.01
<i>m</i> -xylene	1.00 ± 0.01	0.97 ± 0.01	1.06 ± 0.00	0.98 ± 0.01	0.92 ± 0.00
<i>o</i> -xylene	0.94 ± 0.01	0.94 ± 0.00	1.06 ± 0.00	0.96 ± 0.01	0.92 ± 0.02
toluene	0.88 ± 0.01	0.85 ± 0.01	1.05 ± 0.00	0.98 ± 0.01	0.95 ± 0.00
benzene	0.96 ± 0.01	0.92 ± 0.01	1.03 ± 0.01	0.99 ± 0.02	0.87 ± 0.02

Table S3. Fluorescence modulation results of fluorophore **6** with analytes **1-5** in aqueous samples collected from fuel spill sites

Analyte	Cyclodextrin	Downstream	Pipe	Upstream
1	α -CD	1.13 ± 0.01	1.13 ± 0.02	1.12 ± 0.01
1	β -CD	1.15 ± 0.00	1.09 ± 0.05	1.16 ± 0.02
1	Me- β -CD	0.98 ± 0.00	0.97 ± 0.01	0.98 ± 0.01
1	2-HPCD	1.04 ± 0.00	1.06 ± 0.04	1.05 ± 0.01
1	γ -CD	1.11 ± 0.01	1.09 ± 0.01	1.11 ± 0.05
1	PBS	1.15 ± 0.01	1.18 ± 0.01	1.20 ± 0.02
2	α -CD	1.17 ± 0.00	1.26 ± 0.01	1.15 ± 0.01
2	β -CD	1.11 ± 0.04	1.12 ± 0.01	1.09 ± 0.03
2	Me- β -CD	0.94 ± 0.00	0.94 ± 0.01	0.96 ± 0.00
2	2-HPCD	1.04 ± 0.01	1.11 ± 0.00	1.04 ± 0.01
2	γ -CD	1.09 ± 0.03	1.08 ± 0.01	1.06 ± 0.01
2	PBS	1.14 ± 0.03	1.19 ± 0.02	1.16 ± 0.01
3	α -CD	1.32 ± 0.01	1.29 ± 0.01	1.40 ± 0.01
3	β -CD	1.15 ± 0.01	1.19 ± 0.01	1.12 ± 0.02
3	Me- β -CD	0.96 ± 0.01	0.96 ± 0.02	0.95 ± 0.00
3	2-HPCD	1.11 ± 0.00	1.55 ± 0.02	1.17 ± 0.01
3	γ -CD	1.11 ± 0.01	1.16 ± 0.02	1.11 ± 0.01
3	PBS	1.16 ± 0.01	1.25 ± 0.01	1.14 ± 0.02
4	α -CD	1.18 ± 0.00	1.26 ± 0.02	1.14 ± 0.01
4	β -CD	1.08 ± 0.00	1.10 ± 0.01	1.07 ± 0.01
4	Me- β -CD	0.96 ± 0.01	0.96 ± 0.02	0.95 ± 0.00
4	2-HPCD	1.07 ± 0.01	1.12 ± 0.01	1.05 ± 0.00
4	γ -CD	1.06 ± 0.00	1.04 ± 0.01	1.06 ± 0.01
4	PBS	1.11 ± 0.01	1.13 ± 0.01	1.12 ± 0.00
5	α -CD	1.11 ± 0.03	1.16 ± 0.01	1.14 ± 0.02
5	β -CD	1.11 ± 0.01	1.13 ± 0.02	1.09 ± 0.01
5	Me- β -CD	0.97 ± 0.01	1.00 ± 0.02	0.94 ± 0.01
5	2-HPCD	1.05 ± 0.01	1.09 ± 0.00	1.05 ± 0.01
5	γ -CD	1.08 ± 0.00	1.08 ± 0.02	1.08 ± 0.01
5	PBS	1.10 ± 0.01	1.15 ± 0.02	1.12 ± 0.01

SUMMARY TABLE FOR LIMIT OF DETECTION EXPERIMENTS

Table S4. Limit of detection for analytes **1-5** with fluorophore **6** in purified buffer solutions

analyte	fluorophore	host	LOD	LOQ	Equation	R2
m-xylene	BODIPY	α -CD	107.6	265.3	$y = 60.369x + 34374$	0.9999
m-xylene	BODIPY	β -CD	53.3	251.5	$y = 41.836x + 122944$	0.9771
m-xylene	BODIPY	Me- β -CD	a	a	$y = -21.724x + 219660$	0.9989
m-xylene	BODIPY	2-HPCD	25.4	a	$y = -20.964x + 199430$	0.9727
m-xylene	BODIPY	PBS	8.7	304.3	$y = 74.307x + 69635$	0.995
benzene	BODIPY	α -CD	a	180	$y = 18.323x + 102259$	0.9978
benzene	BODIPY	β -CD	184.7	446.3	$y = 22.689x + 99267$	0.9904
benzene	BODIPY	Me- β -CD	a	a	$y = -6.96x + 56294$	0.6863
benzene	BODIPY	2-HPCD	459	1225	$y = 10.496x + 136657$	0.9904
benzene	BODIPY	PBS	a	322.9	$y = 30.256x + 64986$	0.9937
toluene	BODIPY	α -CD	a	684.4	$y = 19.64x + 125163$	0.9813
toluene	BODIPY	β -CD	24.7	129.1	$y = 55.412x + 124609$	0.9995
toluene	BODIPY	Me- β -CD	a	a	$y = -33.994x + 216556$	0.9737
toluene	BODIPY	2-HPCD	72.3	79.9	$y = 32.355x + 33360$	0.9733
toluene	BODIPY	PBS	a	371	$y = 57.301x + 83083$	0.9365
p-xylene	BODIPY	α -CD	123.8	607.1	$y = 22.357x + 40788$	0.9927
p-xylene	BODIPY	β -CD	106.8	685.6	$y = 27.241x + 170075$	0.9999
p-xylene	BODIPY	Me- β -CD	a	a	$y = -16.343x + 273531$	0.9897
p-xylene	BODIPY	2-HPCD	80	a	$y = -45.128x + 244606$	0.9908
p-xylene	BODIPY	PBS	a	128.9	$y = 60.499x + 97392$	0.9865
o-xylene	BODIPY	α -CD	a	44.4	$y = 48.79x + 30222$	0.9999
o-xylene	BODIPY	β -CD	105.5	666.9	$y = 21.302x + 129019$	0.9818
o-xylene	BODIPY	Me- β -CD	a	a	$y = -42.394x + 213215$	0.9813
o-xylene	BODIPY	2-HPCD	a	a	$y = -27.445x + 195701$	0.9925
o-xylene	BODIPY	PBS	a	405.8	$y = 63.796x + 80830$	0.9868

^a Nonsensical values

SUMMARY TABLES FOR ARRAY EXPERIMENTS

Table S5. Results of array generation of analytes **1-5** in purified buffer solution with cyclodextrins as predictors

Jackknifed Classification Matrix

	benzene	m-xylene	o-xylene	p-xylene	toluene	%correct
benzene	4	0	0	0	0	100
m-xylene	0	4	0	0	0	100
o-xylene	0	0	4	0	0	100
p-xylene	0	0	0	4	0	100
toluene	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.515	0.934	1.000	1.000
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Table S6. Results of array generation of analytes **1-5** in downstream sample with cyclodextrins as predictors

Jackknifed Classification Matrix

	Benzene	Toluene	m-xylene	o-xylene	p-xylene	%correct
Benzene	4	0	0	0	0	100
Toluene	0	4	0	0	0	100
m-xylene	0	0	4	0	0	100
o-xylene	0	0	0	4	0	100
p-xylene	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.882	0.987	0.999	1.000
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Table S7. Results of array generation of analytes **1-5** in pipe sample with cyclodextrins as predictors

Jackknifed Classification Matrix

	Benzene	Toluene	m-xylene	o-xylene	p-xylene	%correct
Benzene	4	0	0	0	0	100
Toluene	0	4	0	0	0	100
m-xylene	0	0	4	0	0	100
o-xylene	0	0	0	4	0	100
p-xylene	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.832	0.944	0.995	1.000
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Table S8. Results of array generation of analytes **1-5** in upstream sample with cyclodextrins as predictors

Jackknifed Classification Matrix

	Benzene	Toluene	m-xylene	o-xylene	p-xylene	%correct
Benzene	4	0	0	0	0	100
Toluene	0	4	0	0	0	100
m-xylene	0	0	4	0	0	100
o-xylene	0	0	0	4	0	100
p-xylene	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.765	0.981	0.999	1.000
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Table S9. Results of array generation of aqueous samples with cyclodextrins in purified buffer solution as predictors

Jackknifed Classification Matrix

	Downstream	Pipe	Upstream	%correct
Downstream	4	0	0	100
Pipe	0	4	0	100
Upstream	0	0	4	100
Total	4	4	4	100

Cumulative Proportion of Total Dispersion

0.722	1.000
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Table S10. Results of array generation of aqueous samples with cyclodextrins in pure water as predictors

Jackknifed Classification Matrix

	Downstream	Pipe	Upstream	%correct
Downstream	4	0	0	100
Pipe	0	4	0	100
Upstream	0	0	4	100
Total	4	4	4	100

Cumulative Proportion of Total Dispersion

0.994	1.000
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FLUORESCENCE TABLES FROM AQUEOUS SAMPLES

Table S11. Normalized integration values from blank fluorescence spectra for aqueous fuel site samples

	250 nm	360 nm	460 nm
upstream	0.67 ± 0.00	0.19 ± 0.01	0.18 ± 0.00
pipe	0.94 ± 0.05	0.76 ± 0.22	0.60 ± 0.35
downstream	0.68 ± 0.02	0.21 ± 0.01	0.19 ± 0.00

SUMMARY FIGURES FROM FLUORESCENCE MODULATION EXPERIMENTS

Figure S15. Fluorescence modulation of analyte **1** in purified buffer solution

Figure S16. Fluorescence modulation of analyte **2** in purified buffer solution

Figure S17. Fluorescence modulation of analyte **3** in purified buffer solution

Figure S18. Fluorescence modulation of analyte **4** in purified buffer solution

Figure S19. Fluorescence modulation of analyte **5** in purified buffer solution

Figure S20. Fluorescence modulation of analyte **1** in upstream sample

Figure S21. Fluorescence modulation of analyte 1 in pipe sample

Figure S22. Fluorescence modulation of analyte 1 in downstream sample

Figure S23. Fluorescence modulation of analyte 2 in upstream sample

Figure S24. Fluorescence modulation of analyte 2 in pipe sample

Figure S25. Fluorescence modulation of analyte 2 in downstream sample

Figure S26. Fluorescence modulation of analyte 3 in upstream sample

Figure S27. Fluorescence modulation of analyte **3** in pipe sample

Figure S28. Fluorescence modulation of analyte **3** in downstream sample

Figure S29. Fluorescence modulation of analyte 4 in upstream sample

Figure S30. Fluorescence modulation of analyte 4 in pipe sample

Figure S31. Fluorescence modulation of analyte 4 in downstream sample

Figure S32. Fluorescence modulation of analyte 5 in upstream sample

Figure S33. Fluorescence modulation of analyte 5 in pipe sample

Figure S34. Fluorescence modulation of analyte 5 in downstream sample

SUMMARY FIGURES FOR ARRAY GENERATION EXPERIMENTS

Figure S34. Linear discriminant analysis of fluorescence responses for analytes **1-5** in purified buffer solution with cyclodextrins as predictors

Figure S36. Linear discriminant analysis of fluorescence responses for analytes **1-5** in the downstream sample with cyclodextrins as predictors

Figure S37. Linear discriminant analysis of fluorescence responses for analytes 1-5 in the pipe sample with cyclodextrins as predictors

Figure S38. Linear discriminant analysis of fluorescence responses for analytes 1-5 in the upstream sample with cyclodextrins as predictors

Figure S39. Linear discriminant analysis of fluorescence responses for fluorophore 6 in aqueous fuel spill site samples in purified buffer solutions with cyclodextrins as predictors

Figure S40. Linear discriminant analysis of fluorescence responses for fluorophore 6 in aqueous fuel spill site samples in pure water with cyclodextrins as predictors

SUMMARY FIGURES FOR LIMIT OF DETECTION EXPERIMENTS

Figure S41. Limit of detection of analyte **1** with α -cyclodextrin in purified buffer solution

Figure S42. Limit of detection of analyte **1** with β -cyclodextrin in purified buffer solution

Figure S43. Limit of detection of analyte **1** with methyl- β -cyclodextrin in purified buffer solution

Figure S44. Limit of detection of analyte **1** with 2-hydroxypropyl- β -cyclodextrin in purified buffer solution

Figure S45. Limit of detection of analyte **1** without cyclodextrin in purified buffer solution

Figure S46. Limit of detection of analyte **2** with α -cyclodextrin in purified buffer solution

Figure S47. Limit of detection of analyte **2** with β -cyclodextrin in purified buffer solution

Figure S48. Limit of detection of analyte **2** with methyl- β -cyclodextrin in purified buffer solution

Figure S49. Limit of detection of analyte **2** with 2-hydroxypropyl- β -cyclodextrin in purified buffer solution

Figure S50. Limit of detection of analyte **2** without cyclodextrin in purified buffer solution

Figure S51. Limit of detection of analyte **3** with α -cyclodextrin in purified buffer solution

Figure S52. Limit of detection of analyte **3** with β -cyclodextrin in purified buffer solution

Figure S53. Limit of detection of analyte **3** with methyl-β-cyclodextrin in purified buffer solution

Figure S54. Limit of detection of analyte **3** with 2-hydroxypropyl-β-cyclodextrin in purified buffer solution

Figure S55. Limit of detection of analyte **3** without cyclodextrin in purified buffer solution

Figure S56. Limit of detection of analyte **4** with α -cyclodextrin in purified buffer solution

Figure S57. Limit of detection of analyte 4 with β -cyclodextrin in purified buffer solution

Figure S58. Limit of detection of analyte 4 with methyl- β -cyclodextrin in purified buffer solution

Figure S59. Limit of detection of analyte **4** with 2-hydroxypropyl- β -cyclodextrin in purified buffer solution

Figure S60. Limit of detection of analyte **4** without cyclodextrin in purified buffer solution

Figure S61. Limit of detection of analyte **5** with α -cyclodextrin in purified buffer solution

Figure S62. Limit of detection of analyte **5** with β -cyclodextrin in purified buffer solution

Figure S63. Limit of detection of analyte **5** with methyl-β-cyclodextrin in purified buffer solution

Figure S64. Limit of detection of analyte **5** with 2-hydroxypropyl-β-cyclodextrin in purified buffer solution

Figure S65. Limit of detection of analyte **5** without cyclodextrin in purified buffer solution

CHAPTER 5

Published in *Analytical Letters* **2018**, *Ahead of Print*

Efficient Detection of Phthalate Esters in Human Saliva via Fluorescence Spectroscopy

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Manuscript 5

Efficient Detection of Phthalate Esters in Human Saliva via Fluorescence

Spectroscopy

ABSTRACT

The detection of phthalates in human biological fluids remains an important research objective because it provides an important measure of an individual's exposure to this class of compounds, which have known deleterious health effects. Moreover, the ability to accomplish such detection in fluids that are easy to collect, such as saliva and urine, provides additional practical advantages. Reported herein is the application of cyclodextrin-promoted fluorescence energy transfer and fluorescence modulation to accomplish precisely such detection: the development of sensitive and selective fluorescence-based detection methods for phthalates in saliva, an easily collectable human biological fluid. Such saliva-based detection methods occur with high levels of selectivity (100 % differentiation) and sensitivity (limits of detection as low as 0.089 μM), and provide significant potential in the development of practical phthalate detection devices.

INTRODUCTION

Phthalates are compounds that are of significant concern due to their toxic health effects, particularly as endocrine disruptors.^{1,2} Dibutylphthalate, for example, has been shown to have anti-androgenic effects,³ and diisononylphthalate increases the occurrence of reproductive malformations.⁴ Exposure to phthalates can occur through the use of a broad variety of commercial products,⁵ including fragrance cosmetics,⁶

plastics that have been softened by phthalates,⁷ and a variety of vinyl⁸ and food products.⁹

Current methods for phthalate detection generally rely on gas chromatography-mass spectrometry (GC-MS)^{10,11} or liquid chromatography-mass spectrometry (LC-MS).¹² While such methods have extremely high sensitivity for a broad variety of phthalates, they require significant time for sample preparation and analysis, as well as financial resources for the high cost instrumentation necessary to conduct such analyses and personnel resources for a highly trained instrument operator.^{13,14}

Research in the Levine group has focused on the development of a fundamentally different detection method, using fluorescence-based detection in systems where cyclodextrin can promote favorable intermolecular interactions. In such systems, the presence of the toxicant in close proximity to a high quantum yield fluorophore (where the proximity is facilitated by the cyclodextrin) leads to efficient toxicant-to-fluorophore energy transfer,¹⁵⁻¹⁸ in cases where the toxicant is photophysically active, and toxicant-specific, proximity-induced fluorescence modulation,¹⁹⁻²² in cases where the toxicant is not photophysically active. In both cases, the system relies on favorable interactions between the cyclodextrin, toxicant, and fluorophore, and generates fluorescence response signals that are highly toxicant-specific for broad classes of toxicants in multiple complex environments, and highly sensitive in their ability to detect low concentrations of toxicants.

The detection of phthalates using cyclodextrin-promoted fluorescence modulation has not been reported to date, despite the fact that the phthalates have numerous structural features that are expected to facilitate their favorable interactions

with cyclodextrin.²³ In particular, the hydrophobic components of the phthalates will bind in the hydrophobic cyclodextrin interior,²⁴ whereas the carbonyl moieties will hydrogen bond with one of the cyclodextrin rims, likely the wider one with less steric congestion.²⁵ Fluorescence modulation-based detection of phthalates would obviate many of the challenges associated with mass spectral detection, including the need for costly instrumentation (many portable and inexpensive fluorescence spectrometers exist), and the need to incorporate chromatographic purification prior to mass spectral detection (a result of the fact that every component in a complex mixture generates its own mass spectral signal).

Whether an individual has been exposed to toxicants, as well as the quantity (both at one time and over prolonged time periods), and identity of those toxicants, is important information for medical professionals in assessing that individual's risk of developing exposure-related disease.²⁶ Such exposure is generally determined through detecting toxicants and/or toxicant metabolites in an individual's biological fluids, including in urine²⁷ and breast milk,²⁸ and we have previously reported the ability of cyclodextrin-promoted detection to operate in these environments.^{29,30} The detection of toxicants in saliva using cyclodextrin-promoted detection has not been reported to date, despite the fact that saliva-based detection has a number of attractive features,³¹ including the non-invasive nature of sample collection and the fact that ingested toxicants in saliva will have undergone limited metabolism and will be easier to detect in their native forms.³² One potential complication is that cyclodextrins may undergo hydrolysis by the amylase present in saliva, although literature reports confirm that only

γ -cyclodextrin is likely to undergo such hydrolysis on time scales that are relevant for these detection experiments.³³

Reported herein is the fluorescence detection of a variety of phthalates, in both purified buffer solution and in saliva. This system operates with high sensitivity (micromolar detection limits), selectivity (100% differentiation even between structurally similar analytes), and general applicability for a variety of phthalate esters as well as for binary mixtures of those esters).

EXPERIMENTAL

Materials and Methods

Figure 1. Structures of analytes **1-4**, control analyte **5**, and fluorophores **6-8**

All phthalates and control analytes (compounds **1-5**, Figure 1) were purchased from Sigma-Aldrich chemical company and used as received, unless otherwise noted. All cyclodextrins were purchased from Tokyo Chemical Industry (TCI) and used as received. Fluorophore **6** was synthesized following literature-reported procedures.³⁴ Fluorophores **7** and **8** were purchased from Sigma Aldrich and used as received. Single

donor human saliva was purchased from Innovative Research, Inc. and stored in the freezer until use. ^1H NMR spectra were obtained using a Bruker 300 MHz spectrometer. UV-Visible spectra were obtained using an Agilent 8453 spectrometer equipped with a photodiode array detector. Fluorescence spectra were obtained using a Shimadzu RF-6000 spectrophotofluorimeter with 3.0 nm excitation and emission slit widths. GC-MS measurements were obtained using a Shimadzu GC-MS QP2020 gas chromatograph-mass spectrometer. Computational experiments were performed using Spartan 16 software using energy-minimized conformations.

General Procedure for GC-MS Characterization Experiments

GC-MS sample preparation was conducted following literature-reported procedures.³⁵ In brief, 1 mL of saliva and 2 mL ethyl acetate were added to a glass vial. The vial was shaken for 1 minute, and the organic extract was collected in a separate glass vial. This procedure was repeated two times, and the organic extracts were combined. The samples were concentrated using the rotary evaporator at 53 °C until approximately 200 μL sample volume remained.

All GC-MS measurements were performed using a Shimadzu GC-MS QP2020 gas chromatograph-mass spectrometer following literature-reported procedures.³⁵ The GC-MS operating conditions were as follows: Shimadzu SH-Rxi-5SilMS (30 m x 0.25 mm x 0.25 μm); carrier gas: helium at 1.0 mL/min; oven temperature: 50 °C $\rightarrow$ 50 °C/min to 120 °C (5 min) $\rightarrow$ 10 °C/min to 230 °C $\rightarrow$ 120 °C/min to 280 °C (1 min); injection temperature: 250 °C, splitting ratio: splitless; electron impact ionization mode; MS ion source temperature: 230 °C; interface temperature: 150 °C; total run time: 30 min.

General Procedure for Fluorescence Modulation Experiments

For buffer experiments, 2.5 mL of a 10 mM cyclodextrin solution dissolved in phosphate-buffered-saline (PBS) was added to a quartz cuvette. For saliva experiments, 1.25 mL of a 10 mM cyclodextrin solution dissolved in PBS and 1.25 mL of the saliva sample were combined in a quartz cuvette. A small amount of fluorophore **6-8** (100 μ L, 0.1 mg/mL in methanol) was added, and the solution was excited at the excitation wavelength of the fluorophore (460 nm for fluorophore **6**, 490 nm for fluorophore **7**, and 420 nm for fluorophore **8**). Analytes **1-4** (20 μ L, 1.0 mg/mL solution in methanol) or control analyte **5** were added to the cuvette, and the resulting solution was excited at the excitation wavelength of the fluorophore. The fluorescence emission spectra were integrated versus wavenumber on the X-axis, and the fluorescence modulation was measured by the ratio of integrated fluorescence emission of the fluorophore in the presence of analyte divided by the integrated fluorescence emission of the fluorophore in the absence of the analyte, as shown in Equation 1.

$$\text{Fluorescence modulation} = F/F_0 \quad (1)$$

Where F is the integrated fluorescence emission of the fluorophore in the presence of analyte, and F_0 is the integrated fluorescence emission of the fluorophore in the absence of analyte. All experiments were performed at room temperature (~ 22 $^{\circ}$ C). Control experiments were performed in which 0 mM cyclodextrin solution in PBS was used in place of 10 mM cyclodextrin in PBS.

For mixture experiments, the above procedure was repeated; however, 1:1 (vol/vol) mixtures of analytes (10 μ L, 1.0 mg/mL in methanol) were added to the

cyclodextrin or saliva-cyclodextrin solutions, and the fluorescence modulation values were calculated for fluorophores **6-8** following Equation 1.

General Procedure for Limit of Detection Experiments

Limit of detection experiments were performed following literature-reported procedures.³⁶ For buffer experiments, 2.5 mL of a 10 mM cyclodextrin solution dissolved in phosphate-buffered-saline (PBS) was added to a quartz cuvette. For saliva experiments, 1.25 mL of a 10 mM cyclodextrin solution dissolved in PBS and 1.25 mL of the saliva sample were combined in a quartz cuvette. 100 μ L of fluorophore **6** (0.1 mg/mL in methanol) was added to the solution and excited six times at 460 nm. Next, 5 μ L of analyte (1.0 mg/mL in methanol) was added, and again the solution was excited at fluorophore **6**'s excitation wavelength. Six repeat measurements were taken. This step was repeated for 10 μ L of analyte, 15 μ L of analyte, 20 μ L of analyte, 25 μ L of analyte, 30 μ L of analyte, 35 μ L of analyte, and 40 μ L of analyte.

All of the fluorescence emission spectra were integrated vs. wavenumber on the X-axis, and the calibration curves were generated. The curves plotted the analyte concentration in μ M on the X-axis, and the fluorescence modulation ratio on the Y-axis. The curve was fitted to a straight line and the equation of the line was determined. The limit of detection was calculated according to Equation 2.

$$\text{LOD} = 3(\text{SD}_{\text{blank}})/m \quad (2)$$

Where SD_{blank} is the standard deviation of the blank sample and m is the slope of the calibration curve. In cases where the slope of the trend line was negative, the absolute value of the slope was used to calculate the LOD. In all cases, the LOD was calculated in μ M.

General Procedure for Array Generation Experiments

Array analysis was performed using SYSTAT 13 statistical computing software with the following settings: (a) Classical discriminant analysis; (b) Grouping variable: analytes; (c) Predictors: fluorophores; and (d) Long-range statistics: Mahal.

RESULTS AND DISCUSSION

Saliva Characterization

The human saliva sample was characterized using GC-MS to determine the presence of inherent chemicals components, including those that are typically found in saliva and those that may be a result of toxicant exposure. Typical saliva components found in the sample include long-chain hydrocarbons typically found in human saliva (Figure 2).³⁷ Moreover, traces of caffeine, commonly found in a wide range of food and beverages,^{38,39} and ditridecyl phthalate, commonly used as a plasticizer in housing insulation and automobile insulation,⁴⁰ were also found in the saliva, and indicate that the single anonymous donor may have consumed caffeine and been exposed to phthalate-containing insulation prior to donating his/her saliva.

Figure 2. GC-MS spectrum of human saliva sample

Cyclodextrin Selection

Cyclodextrins selected for this research include α -cyclodextrin and β -cyclodextrin, dissolved in phosphate buffered saline (PBS, buffered at pH 7.4), as well as a control solution of PBS with no cyclodextrin present. These hosts were selected because they are known to bind at least one of the high quantum yield fluorophores, are readily available, have structural features that will facilitate their interactions with phthalates (via hydrophobic association via and/or intermolecular hydrogen bonding), and are not broken down by the amylase found in saliva. γ -Cyclodextrin, by contrast, was excluded from these studies due its strong propensity for rapid amylase-induced degradation, despite the fact that it has been shown to facilitate cyclodextrin-promoted fluorescence detection under a broad variety of conditions.³³

Fluorophore Selection

The fluorophores selected for this research include three common classes of fluorophores: BODIPY, Rhodamine, and Coumarin. Fluorophores **6-8** have been widely used by our group for cyclodextrin-promoted fluorescence-based detection. Moreover, all three of these fluorophores have high quantum yields, good photostability, and have been used in a wide variety of detection schemes.⁴¹⁻⁴³

Analyte Selection

The analytes targeted for detection include phthalate esters that are most commonly found in commercial products, including cosmetics, personal care products, and plasticizers.^{44,45} Analyte **1** is commonly found in insect repellent.⁴⁶ Analyte **2** has been widely used in perfume fragrances.⁴⁷ Analyte **3** can be found in nail lacquer.⁴⁸ Analyte **4** is commonly found in polyvinyl chloride (PVC) pipes.⁴⁹ Because individuals are exposed to these commercial products on a regular basis in their daily lives, there is significant benefit to the individuals, their physicians, and scientific researchers to monitoring and quantifying such exposure to better understand the complex relationship between toxicant exposure and the development of exposure-related disease. Measuring the presence of these highly common phthalates in saliva provides one important way to measure such exposure.

Fluorescence Modulation

Each cyclodextrin-fluorophore combination was used to enable the detection of specific phthalate esters in both purified buffer systems and human saliva samples. Micromolar concentrations of analytes **1-4** or control analyte **5** were added to each saliva-cyclodextrin-fluorophore or buffer-cyclodextrin-fluorophore combination, and

the degree of fluorescence modulation of fluorophores **6-8** in the presence and absence of analyte was calculated using Equation 1. Each of the three fluorophores selected for analysis displays different behaviors and trends in the modulation results.

Table 1. Fluorescence modulation values for analytes with BODIPY^a

Analyte	Buffer			Saliva		
	α -CD	β -CD	PBS	α -CD	β -CD	PBS
1	0.98 $\pm$ 0.02	1.15 $\pm$ 0.02	1.43 $\pm$ 0.03	1.15 $\pm$ 0.02	1.09 $\pm$ 0.02	1.21 $\pm$ 0.04
2	1.12 $\pm$ 0.04	1.01 $\pm$ 0.00	1.07 $\pm$ 0.01	1.06 $\pm$ 0.06	0.99 $\pm$ 0.02	1.11 $\pm$ 0.02
3	1.15 $\pm$ 0.04	1.27 $\pm$ 0.0	1.77 $\pm$ 0.05	1.12 $\pm$ 0.02	1.11 $\pm$ 0.03	1.20 $\pm$ 0.03
4	2.62 $\pm$ 0.06	3.96 $\pm$ 0.12	2.16 $\pm$ 0.04	1.95 $\pm$ 0.02	1.69 $\pm$ 0.15	1.77 $\pm$ 0.15
5	0.96 $\pm$ 0.01	1.19 $\pm$ 0.08	1.14 $\pm$ 0.04	0.99 $\pm$ 0.01	1.13 $\pm$ 0.03	0.96 $\pm$ 0.08

^aAll results represent an average of results from four trials for each sample. Fluorescence modulation values were calculated using Equation 1.

BODIPY (fluorophore **6**) shows the highest changes in fluorescence modulation with the introduction of the target analytes (Table 1). This is likely due to the fact that the predominant way in which BODIPY interacts with cyclodextrin is also one of the key ways in which phthalate esters interact with cyclodextrin, via intermolecular hydrogen bonding of the small molecule with the cyclodextrin rim.^{50,51} Introduction of the analyte therefore results in significant disruption of the BODIPY-cyclodextrin association, which is manifested in the fact that the BODIPY emission spectra changes so dramatically.

Figure 3. Fluorescence changes of BODIPY upon introduction of (A) no analyte in buffer, (B) dimethyl phthalate in buffer, (C) no analyte in saliva, and (D) dimethyl phthalate in saliva. The black line represents α -cyclodextrin, the red line represents β -cyclodextrin, and the blue line represents phosphate buffered saline (PBS). Analytical conditions: BODIPY (100 μ L, 0.1 mg/mL in THF); dimethyl phthalate (20 μ L, 1.0 mg/mL in THF); excitation wavelength: 460 nm; emission range: 470-800 nm; excitation and emission slit widths: 3.0 nm.

Similar intermolecular interactions of BODIPY and phthalates with the cyclodextrin hosts also explains why unique emission characteristics are observed for each of the different cyclodextrin hosts: because the BODIPY-cyclodextrin association and the disruption of such association is so key for determining the unique modulation responses, changes to the identity of the cyclodextrin result in significant changes in the BODIPY's microenvironment, which strongly affects the resulting observable fluorescence emission before and after analyte addition (Figure 3).

The fluorescence emission spectrum of Rhodamine 6G (fluorophore **7**) displayed little change with the addition of analyte, for all analytes and all cyclodextrin combinations tested (Table 2).

Table 2. Fluorescence modulation values for analytes with Rhodamine 6G^a

Analyte	Buffer			Saliva		
	α -CD	β -CD	PBS	α -CD	β -CD	PBS
1	0.99 $\pm$	1.00 $\pm$	0.98 $\pm$	0.98 $\pm$	1.01 $\pm$	0.98 $\pm$
	0.00	0.00	0.00	0.00	0.00	0.00
2	0.99 $\pm$	0.99 $\pm$	0.98 $\pm$	0.99 $\pm$	1.00 $\pm$	0.99 $\pm$
	0.00	0.00	0.00	0.00	0.00	0.00
3	1.00 $\pm$	0.99 $\pm$	0.99 $\pm$	1.00 $\pm$	1.00 $\pm$	0.98 $\pm$
	0.00	0.00	0.00	0.00	0.01	0.00
4	1.00 $\pm$	1.00 $\pm$	0.98 $\pm$	1.00 $\pm$	1.02 $\pm$	0.98 $\pm$
	0.00	0.00	0.00	0.00	0.00	0.00
5	0.97 $\pm$	1.00 $\pm$	0.97 $\pm$	0.99 $\pm$	0.99 $\pm$	0.99 $\pm$
	0.00	0.00	0.00	0.00	0.00	0.00

^aAll results represent an average of results from four trials for each sample. Fluorescence modulation values were calculated using Equation 1.

These results indicate a limited association between the Rhodamine 6G and the analytes, likely as a result of the twisted biphenyl axis that precludes close-range intermolecular interactions.⁵² Some association between fluorophore **7** and the cyclodextrin hosts is likely, however, as indicated by the fact that the fluorescence intensity of fluorophore **7** is higher in the presence of β -cyclodextrin compared to the emission in cyclodextrin-free (i.e. PBS) solution (Figure 4). Intermediate values of fluorescence emission were observed in the presence of α -cyclodextrin, indicating some (albeit limited) association likely occurs.

Figure 4. Fluorescence emission of Rhodamine 6G upon introduction of (A) no analyte in buffer, (B) diethyl phthalate in buffer, (C) no analyte in saliva, and (D) diethyl phthalate in saliva. The black line represents α -cyclodextrin, the red line represents β -cyclodextrin, and the blue line represents phosphate buffered saline (PBS). Analytical conditions: Rhodamine (100 μ L, 0.1 mg/mL in THF); diethyl phthalate (20 μ L, 1.0 mg/mL in THF); excitation wavelength: 490 nm; emission range: 500-800 nm; excitation and emission slit widths: 3.0 nm.

The fluorescence emission spectrum of Coumarin 6 (fluorophore **8**) displays excimer-like emission peaks, both in the presence of α -cyclodextrin and in the absence of any cyclodextrin (pure PBS) (select results shown in Figure 5).

Figure 5. Fluorescence changes of Coumarin 6 upon introduction of dibutyl phthalate with (A) α -cyclodextrin, (B) β -cyclodextrin, and (C) phosphate buffered saline (PBS). The black line represents buffer and the red line represents saliva. Analytical conditions: Coumarin 6 (100 μ L, 0.1 mg/mL in THF); dibutyl phthalate (20 μ L, 1.0 mg/mL in THF); excitation wavelength: 420 nm; emission range: 430-800 nm; excitation and emission slit widths: 3.0 nm.

This indicates that fluorophore **8** does not bind in the α -cyclodextrin cavity, and instead self-associates in a mostly aqueous solvent environment, resulting in the observed excimer emission. In the presence of β -cyclodextrin, by contrast, no excimer peaks are observed, which is a direct result of the strong binding of fluorophore **8** in the β -cyclodextrin cavity (binding affinity $\approx 10,000$ M⁻¹).⁵³ Moreover, the strong binding also results in limited changes to the fluorescence of fluorophore **8** in β -cyclodextrin with the introduction of phthalate analytes, which is a result of the inability of the phthalates to displace the strongly bound fluorophore from the cyclodextrin cavity.

In addition to the fluorophore-specific trends discussed above, analyte-specific trends were also observed. In particular, the addition of diisononyl phthalate (analyte **4**) resulted in the highest degree of fluorescence modulation with fluorophores **6-8** and most dramatic changes in fluorescence emission compared to the other analytes (Figure 6).

Figure 6. Fluorescence changes of (A) BODIPY, (B) Rhodamine 6G, and (C) Coumarin 6 upon introduction of diisononyl phthalate in saliva. The black line represents α -cyclodextrin, the red line represents β -cyclodextrin, and the blue line represents phosphate buffered saline (PBS). Analytical conditions: BODIPY, Rhodamine, and Coumarin 6 (100 μ L, 0.1 mg/mL in THF); diisononyl phthalate (20 μ L, 1.0 mg/mL in THF); excitation wavelength: BODIPY = 460 nm, Rhodamine 6G = 490 nm, and Coumarin 6 = 420 nm; emission range: BODIPY = 470-800 nm, Rhodamine 6G = 500-800 nm, and Coumarin 6 = 430-800 nm; excitation and emission slit widths = 3.0 nm.

This is likely a result of the long, hydrophobic isononyl chains binding in the cyclodextrin cavity and promoting strong cyclodextrin-analyte association, as well as significant disruption of the cyclodextrin-fluorophore association. In support of this explanation, literature-reported binding constants of the analytes in β -cyclodextrin follow the trend that longer alkyl chains result in strong binding affinities, due to the ability of the alkyl chains to bind in the interior cyclodextrin cavity via hydrophobically-induced complexation (Table 3).

Of note, we also tested for any observable effects that could be attributed to the solvent used to dissolve the phthalate analytes, methanol, in the absence of any added analyte. As expected, only minimal changes in the fluorescence spectra with the introduction of small amounts of methanol were observed. Due to the ability of methanol to engage in substantial hydrogen bonding with the heteroaromatic fluorophores and to perturb the local microenvironment, we observed small changes in

the fluorescence emission of the fluorophore with the addition of methanol as the control analyte.^{54,55}

Table 3. Literature-reported binding constants for analytes with β -cyclodextrin^a

Analyte	Binding Constant (M^{-1})
1	82
2	107
3	1160
5	<i>b</i>

^aBinding constant values obtained from Hattori 1999. ^bNo literature-reported binding constant available.

These interactions are fundamentally different from the interactions between the phthalate analytes and the fluorophores, both in terms of the intermolecular forces that underlie such interactions as well as in the magnitude of the fluorescence emission changes. Our use of the solvent methanol, rather than tetrahydrofuran which was used as the solvent in our previous fluorescence studies, was driven by the need to ensure full solubility of all analytes, while requiring full miscibility with the majority aqueous solvent system that is required for the requisite hydrophobically-driven cyclodextrin binding.⁵⁶

Limit of Detection

The sensitivity of the system was determined by calculating LODs for all buffer- β -cyclodextrin-fluorophore **6**-analyte and saliva- β -cyclodextrin-fluorophore **6**-analyte combinations following literature-reported procedures,³⁶ and selected results of these studies are highlighted in Table 4.

Table 4. Limits of detection for analytes with BODIPY and β -cyclodextrin in buffer and saliva^a

Analyte	LOD in Buffer (mg/L)	LOD in Saliva (mg/L)	Exposure Limit (mg/L)^b
1	1.68 $\pm$ 0.32	3.31 $\pm$ 0.21	160
2	0.49 $\pm$ 0.01	0.62 $\pm$ 0.04	60
3	0.28 $\pm$ 0.01	0.41 $\pm$ 0.04	5
4	0.037 $\pm$ 0.001	0.18 $\pm$ 0.04	<i>c</i>

^aLimits of detection were calculated using the procedures in Cheng 2016; see Electronic Supporting Information for more details. Errors are shown with enough significant figures to accurately capture the errors ^b Exposure limits from National Service Center for Environmental Publications 1978; ^c No established exposure limit currently exists.

In general, LODs for analytes in saliva were slightly higher, reflecting slightly worse sensitivities, than those measured in purified buffer systems. This is likely due to the competitive binding of saliva components, such as long-chain alkanes, caffeine, or the inherent phthalate compound ditridecyl phthalate, with cyclodextrin. In every case, the calculated limits of detection were significantly below literature-reported limits of concern for these compounds. These low limits of detection highlight the extreme sensitivity of this cyclodextrin-promoted fluorescence modulation-based detection method in environments containing ppm-levels of phthalates. However, phthalates, if found in saliva, exist at ppb-range concentrations.⁵⁷ Current work in our laboratory is dedicated towards optimizing our detection method to detect phthalates at even lower concentrations.

Array Generation

The selectivity of this system was determined by creating statistical arrays using fluorescence modulation results to differentiate between structurally similar analytes in

both purified buffer systems and in saliva samples. This array-based analysis showed 100% differentiation between analytes **1-5** in buffer and saliva for all cyclodextrin-fluorophore combinations, with a specific example of such differentiation highlighted in Figure 7.

Figure 7. Array-based detection of analytes with β -cyclodextrin in buffer and saliva using BODIPY, Rhodamine 6G, and Coumarin 6 as predictors

The response patterns show well-separated signals between the same analytes in buffer and saliva (i.e. analyte **1** in buffer has a signal that is well separated from analyte **1** in saliva), and overall, analytes in saliva are grouped separately from those measured in buffer. A specific example includes the grouping of analyte **2** in saliva with analyte **3** in saliva and analyte **2** in buffer with analyte **3** in buffer, rather than analyte **2** in both samples grouping together.

Additionally, we can also use our statistical arrays to differentiate between different concentrations of analytes (Figure 8).

Figure 8. Array-based detection of various concentrations of dibutyl phthalate with β -cyclodextrin in (A) buffer and (B) saliva using BODIPY, Rhodamine 6G, and Coumarin 6 as predictors

Figure 8 shows an array generated using different concentrations of analyte **3** with β -cyclodextrin in buffer and saliva. Interestingly, both the buffer and saliva quantitative arrays led to 100% differentiation between different concentrations of analyte **4**. This selectivity builds on the high sensitivity of our system to be able to induce measurable changes in fluorescence emission of fluorophores with very small changes in analyte concentration. This quantitative array provides both the concentration and the identity of the analyte and highlights the potential for the use of statistical arrays to identify and quantify analytes in unknown samples.

Mixture Experiments

In complex biological fluids, such as saliva, oftentimes there are several toxicants present in one sample, which can complicate the accurate detection of analytes. To address the question of toxicant detection in complex mixtures, we tested binary mixtures of phthalates using cyclodextrin-promoted fluorescence modulation

and found that 100% differentiation between binary mixtures of analytes was obtained (Figure 9).

Figure 9. Array-based detection of mixtures of analytes in the presence of (A) α -cyclodextrin; (B) β -cyclodextrin, and (C) PBS using fluorophores BODIPY, Rhodamine 6G, and Coumarin 6 as predictors

Of note, the visual response patterns for analyte mixtures varied depending on the identity of the cyclodextrin hosts. Grouping of analytes is dependent upon cyclodextrin host because the larger β -cyclodextrin cavity may allow binding or association of both analytes, while the smaller α -cyclodextrin cavity may only allow limited association of one analyte in the mixture. In the presence of α -cyclodextrin (Figure 9A), analyte mixtures of analyte **1** and analyte **2**, analyte **1** and analyte **3** and analyte **2** and analyte **3** group closely together. Analytes **3** and **4** are extremely well-separated from the other analyte mixtures in the presence of α -cyclodextrin. In β -cyclodextrin (Figure 9B), analyte mixtures containing analytes **1** and **2** and analytes **1** and **4** group closely together. Analytes **1** and **3**, analytes **2** and **3**, and analytes **3** and **4** are well-separated in the presence of β -cyclodextrin. In the presence of PBS (Figure 9C), no two analyte mixtures are particularly close, each analyte mixture signal is well-separated. Current work in our laboratory is focused on expanding this array-based detection to include ternary and quaternary mixtures of analytes.

CONCLUSIONS

In conclusion, reported herein is the use of cyclodextrin-promoted fluorescence modulation for the detection of phthalate esters in human saliva. This method is selective (100% successful in differentiating structurally similar compounds), sensitive (sub-micromolar detection limits), and generally applicable (for mixtures of analytes). The high selectivity, sensitivity, and general applicability show potential for the development of rapid, on-site detection devices for phthalate esters.

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Efficient Detection of Phthalate Esters in Human Saliva via Fluorescence

Spectroscopy

ANALYTE DETAILS

Figure S1. Structures of analytes **1-4**, control analyte **5**, and fluorophores **6-8****Table S1.** Final solution concentrations of analytes and fluorophores

Compound	Concentration (mg/mL) in methanol	Volume (μ L)	Final Concentration (μ M)
1	1.0	20	39.6
2	1.0	20	34.6
3	1.0	20	27.6
4	1.0	20	18.4
5	1.0	20	240.1
6	0.1	100	9.5
7	0.1	100	8.1
8	0.1	100	11.0

SYNTHESIS OF FLUOROPHORE 6

The synthesis of BODIPY **6** was performed according to literature-reported procedures:

REFERENCE: Shepherd, J. L.; Kell, A.; Chung, E.; Sinclair, C. W.; Workentin, M. S.; Bizzotto, D. *J. Am. Chem. Soc.* **2004**, *126*, 8329-8335.

Reaction 1:

Figure S2. Synthetic pathway to structure **S5**

Procedure: 2.0 grams of 11-bromoundecanoic acid **S1** (7.54 mmol, 1.0 eq.) was combined with 2 drops of *N,N*-dimethylformamide in 40 mL of dichloromethane. 1.0 gram of oxalyl chloride **S2** (7.88 mmol, 1.05 eq.) was dissolved in 5.0 mL of dichloromethane and added dropwise. The reaction mixture was stirred for one hour, then the crude mixture was concentrated on the rotary evaporator and dried on a vacuum overnight to remove any unreacted oxalyl chloride. The resulting acid chloride **S3** was dissolved in 50 mL of dichloromethane. 0.772 mL of 2,4-dimethylpyrrole **S4** (7.50 mmol, 0.99 eq.) was dissolved in 5.0 mL of dichloromethane and added to the reaction mixture. The resulting reaction mixture was heated to reflux for 3 hours under a nitrogen atmosphere, during which time the mixture became a dark red color. After three hours, the reaction mixture was cooled to room temperature and solvent was removed on the rotary evaporator until approximately 5.0 mL of the dichloromethane solution remained. 200 mL of *n*-hexanes were added to the flask, and the mixture was cooled overnight in the freezer at -20 °C. The hexanes were decanted from the insoluble oil and precipitate.

The resulting crude product was dissolved in 75 mL of toluene and heated to 80 °C. 1.0 mL of triethylamine (7.17 mmol, 0.95 eq.) was added and the solution immediately turned light yellow. 1.0 mL of boron trifluoride etherate (8.10 mmol, 1.07 eq.) was then added and the reaction mixture was stirred at 80 °C for 30 minutes, during which time the color of the mixture darkened and became fluorescent. The reaction mixture was cooled to room temperature, and the product was extracted 3 times with brine (50 mL each time). The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography (1:1 dichloromethane: hexanes) to yield the desired product in 28% yield (comparable to the literature-reported 24% yield).

Reaction 2:

Figure S3. Synthetic pathway to structure **S7**

Procedure: Compound **S5** (0.968 g, 2.07 mmol, 1.0 eq.) and compound **S6** (0.27 grams, 2.36 mmol, 1.14 eq.) were dissolved in 50 mL of acetone. The reaction mixture was heated to reflux for two hours. After two hours, the reaction mixture was cooled to room temperature, acetone was removed, and the crude solid was re-dissolved in dichloromethane and washed with water. The organic extract was dried over sodium sulfate, filtered and concentrated, to yield compound **S7** in 97% yield (0.932 grams).

Reaction 3:

Figure S4. Synthetic pathway to compound **6**

Procedure: Compound **S7** (0.932 grams, 2.01 mmol, 1.0 eq.) was dissolved in 150 mL of anhydrous ethanol that was purged with nitrogen. Potassium carbonate was added, and the reaction mixture was warmed to 30 °C. The reaction mixture was stirred under nitrogen for 4 hours at 30 °C. The contents of the flask were poured over 40 mL of aqueous saturated ammonium chloride, at which point the solution turned bright orange. The product was extracted with dichloromethane and washed several times with water. The organic layer was dried over sodium sulfate, filtered, and concentrated. The product was purified via flash chromatography (1:1 dichloromethane: hexanes) to yield compound **6** in 76% yield (674 mg).

¹H NMR OF FLUOROPHORE 6

Figure S5. ¹H NMR spectrum of fluorophore 6

SUMMARY TABLE FOR FLUORESCENCE MODULATION EXPERIMENTS

Table S2. Fluorescence modulation results for analytes in buffer and saliva

Cyclodextrin	Analyte	Fluorophore 6		Fluorophore 7		Fluorophore 8	
		Buffer	Saliva	Buffer	Saliva	Buffer	Saliva
α -CD	1	0.98 ± 0.02	1.15 ± 0.02	0.99 ± 0.00	0.98 ± 0.00	1.72 ± 0.06	0.77 ± 0.07
α -CD	2	1.12 ± 0.04	1.06 ± 0.06	0.99 ± 0.00	0.99 ± 0.00	1.04 ± 0.04	0.97 ± 0.02
α -CD	3	1.15 ± 0.04	1.12 ± 0.02	1.00 ± 0.00	1.00 ± 0.00	1.08 ± 0.08	0.95 ± 0.03
α -CD	4	2.62 ± 0.06	1.95 ± 0.02	1.00 ± 0.00	1.00 ± 0.00	1.72 ± 0.06	1.82 ± 0.03
α -CD	5	0.96 ± 0.01	0.99 ± 0.01	0.97 ± 0.00	0.99 ± 0.00	0.97 ± 0.08	0.94 ± 0.02
β -CD	1	1.15 ± 0.02	1.09 ± 0.02	1.00 ± 0.00	1.01 ± 0.00	1.00 ± 0.00	0.99 ± 0.00
β -CD	2	1.01 ± 0.00	0.99 ± 0.02	0.99 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.01 ± 0.00
β -CD	3	1.27 ± 0.01	1.11 ± 0.03	0.99 ± 0.00	1.00 ± 0.01	1.00 ± 0.00	1.00 ± 0.00
β -CD	4	3.96 ± 0.12	1.69 ± 0.15	1.00 ± 0.00	1.02 ± 0.00	1.05 ± 0.00	1.09 ± 0.00
β -CD	5	1.19 ± 0.08	1.13 ± 0.03	1.00 ± 0.00	0.99 ± 0.00	0.99 ± 0.00	1.00 ± 0.00
PBS	1	1.43 ± 0.03	1.21 ± 0.04	0.98 ± 0.00	0.98 ± 0.00	1.12 ± 0.03	0.94 ± 0.07
PBS	2	1.07 ± 0.01	1.11 ± 0.02	0.98 ± 0.00	0.99 ± 0.00	0.92 ± 0.03	1.12 ± 0.05
PBS	3	1.77 ± 0.05	1.20 ± 0.03	0.99 ± 0.00	0.98 ± 0.00	1.11 ± 0.02	0.96 ± 0.02
PBS	4	2.16 ± 0.04	1.77 ± 0.15	0.98 ± 0.00	0.98 ± 0.00	2.26 ± 0.04	2.39 ± 0.08
PBS	5	1.14 ± 0.04	0.96 ± 0.08	0.97 ± 0.00	0.99 ± 0.00	0.94 ± 0.02	0.96 ± 0.01

SUMMARY TABLE FOR LIMIT OF DETECTION EXPERIMENTS

Table S3. Limits of detection for analytes **1-4** with β -cyclodextrin and fluorophore **6**

Analyte	Matrix	Fluorophore	Equation	R ²	LOD (μ M)
1	Buffer	6	$y = 0.0033x + 1.0568$	0.9938	8.63 ± 1.63
	Saliva	6	$y = 0.0027x + 1.0765$	0.9091	17.06 ± 1.11
2	Buffer	6	$y = 0.0083x + 0.9858$	0.9807	2.22 ± 0.02
	Saliva	6	$y = 0.0061x + 1.0103$	0.9896	2.78 ± 0.19
3	Buffer	6	$y = 0.0094x + 1.0153$	0.9968	1.02 ± 0.02
	Saliva	6	$y = 0.0067x + 1.0391$	0.9803	1.46 ± 0.16
4	Buffer	6	$y = 0.0565x + 0.6971$	0.9920	0.09 ± 0.00
	Saliva	6	$y = 0.0499x + 1.0748$	0.9862	0.42 ± 0.09

SUMMARY TABLES FOR ARRAY GENERATION EXPERIMENTS

Table S4. Results of array generation in buffer with α -cyclodextrin

Jackknifed Classification Matrix

	Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	%correct
Analyte 1	4	0	0	0	0	100
Analyte 2	0	4	0	0	0	100
Analyte 3	0	0	4	0	0	100
Analyte 4	0	0	0	4	0	100
Analyte 5	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.976	0.997	1.000
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Table S5. Results of array generation in buffer with β -cyclodextrin

Jackknifed Classification Matrix

	Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	%correct
Analyte 1	4	0	0	0	0	100
Analyte 2	0	4	0	0	0	100
Analyte 3	0	0	4	0	0	100
Analyte 4	0	0	0	4	0	100
Analyte 5	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.935	0.999	1.000
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Table S6. Results of array generation in buffer without cyclodextrin

Jackknifed Classification Matrix

	Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	%correct
Analyte 1	4	0	0	0	0	100
Analyte 2	0	4	0	0	0	100
Analyte 3	0	0	4	0	0	100
Analyte 4	0	0	0	4	0	100
Analyte 5	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.887	0.992	1.000
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Table S7. Results of array generation in saliva with α -cyclodextrin

Jackknifed Classification Matrix

	Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	%correct
Analyte 1	4	0	0	0	0	100
Analyte 2	0	4	0	0	0	100
Analyte 3	0	0	4	0	0	100
Analyte 4	0	0	0	4	0	100
Analyte 5	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

1.000	1.000	1.000
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Table S8. Results of array generation in saliva with β -cyclodextrin

Jackknifed Classification Matrix

	Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	%correct
Analyte 1	4	0	0	0	0	100
Analyte 2	0	4	0	0	0	100
Analyte 3	0	0	4	0	0	100
Analyte 4	0	0	0	4	0	100
Analyte 5	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.992	0.999	1.000
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Table S9. Results of array generation in saliva without cyclodextrin

Jackknifed Classification Matrix

	Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	%correct
Analyte 1	4	0	0	0	0	100
Analyte 2	0	4	0	0	0	100
Analyte 3	0	0	4	0	0	100
Analyte 4	0	0	0	4	0	100
Analyte 5	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.948	0.996	1.000
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SUMMARY TABLE FOR GC-MS EXPERIMENTS

Table S10. Results of GC-MS characterization of saliva

Sample Type	Retention Time (min)	NIST Compound ID
Doped Standard	6.73	Dimethyl phthalate
	7.29	Diethylphthalate
	8.56	Dibutylphthalate
Undoped Sample	16.15	Caffeine
	18.55	2-methylnonadecane
	19.40	7-n-hexyleicosane
	20.40	9-octylheptadecane
	21.65	10-methyleicosane
	23.25	Bis(tridecyl)phthalate
	24.45	7-methyldocosane
	24.80	11-decyltetracosane

SUMMARY TABLES FOR MIXTURE FLUORESCENCE MODULATION EXPERIMENTS

Table S11. Fluorescence modulation results for analyte mixtures in buffer and saliva

Cyclodextrin	Analyte	Analyte	Fluorophore 6		Fluorophore 7		Fluorophore 8	
			Buffer	Saliva	Buffer	Saliva	Buffer	Saliva
α -CD	1	2	0.99 $\pm$ 0.01	1.10 $\pm$ 0.02	1.00 $\pm$ 0.00	0.98 $\pm$ 0.00	0.98 $\pm$ 0.04	0.25 $\pm$ 0.02
α -CD	1	3	1.06 $\pm$ 0.01	1.07 $\pm$ 0.03	0.99 $\pm$ 0.00	0.99 $\pm$ 0.00	2.88 $\pm$ 0.10	0.28 $\pm$ 0.02
α -CD	1	4	1.73 $\pm$ 0.05	1.52 $\pm$ 0.05	0.99 $\pm$ 0.00	1.01 $\pm$ 0.00	1.30 $\pm$ 0.05	1.44 $\pm$ 0.01
α -CD	2	3	1.03 $\pm$ 0.01	1.20 $\pm$ 0.03	0.99 $\pm$ 0.00	0.99 $\pm$ 0.00	0.98 $\pm$ 0.03	0.24 $\pm$ 0.02
α -CD	2	4	1.63 $\pm$ 0.02	1.62 $\pm$ 0.04	1.00 $\pm$ 0.00	1.01 $\pm$ 0.00	1.48 $\pm$ 0.01	1.52 $\pm$ 0.01
α -CD	3	4	1.10 $\pm$ 0.01	1.20 $\pm$ 0.03	0.99 $\pm$ 0.00	1.01 $\pm$ 0.00	1.99 $\pm$ 0.05	1.13 $\pm$ 0.08
β -CD	1	2	1.02 $\pm$ 0.01	1.12 $\pm$ 0.01	0.99 $\pm$ 0.00	0.99 $\pm$ 0.00	0.99 $\pm$ 0.00	0.99 $\pm$ 0.00
β -CD	1	3	1.07 $\pm$ 0.01	1.19 $\pm$ 0.03	0.98 $\pm$ 0.00	0.99 $\pm$ 0.00	1.00 $\pm$ 0.00	0.98 $\pm$ 0.00
β -CD	1	4	1.64 $\pm$ 0.02	1.38 $\pm$ 0.07	0.99 $\pm$ 0.00	1.01 $\pm$ 0.00	1.02 $\pm$ 0.00	1.03 $\pm$ 0.00
β -CD	2	3	1.19 $\pm$ 0.01	1.08 $\pm$ 0.02	0.99 $\pm$ 0.00	1.00 $\pm$ 0.00	0.99 $\pm$ 0.00	0.99 $\pm$ 0.00
β -CD	2	4	1.48 $\pm$ 0.01	1.46 $\pm$ 0.04	0.99 $\pm$ 0.00	1.01 $\pm$ 0.00	1.03 $\pm$ 0.00	1.02 $\pm$ 0.00
β -CD	3	4	1.50 $\pm$ 0.03	1.47 $\pm$ 0.08	0.99 $\pm$ 0.00	1.00 $\pm$ 0.00	1.02 $\pm$ 0.00	1.02 $\pm$ 0.00
PBS	1	2	1.46 $\pm$ 0.08	1.12 $\pm$ 0.03	0.98 $\pm$ 0.00	0.99 $\pm$ 0.00	0.93 $\pm$ 0.04	0.87 $\pm$ 0.05
PBS	1	3	1.63 $\pm$ 0.03	1.12 $\pm$ 0.01	0.97 $\pm$ 0.00	1.00 $\pm$ 0.00	0.98 $\pm$ 0.06	0.70 $\pm$ 0.10
PBS	1	4	1.34 $\pm$ 0.03	1.33 $\pm$ 0.08	0.99 $\pm$ 0.00	0.99 $\pm$ 0.00	1.41 $\pm$ 0.12	1.75 $\pm$ 0.02
PBS	2	3	1.52 $\pm$ 0.03	1.04 $\pm$ 0.05	0.98 $\pm$ 0.00	0.99 $\pm$ 0.00	0.89 $\pm$ 0.02	0.93 $\pm$ 0.03
PBS	2	4	1.17 $\pm$ 0.02	1.23 $\pm$ 0.07	0.99 $\pm$ 0.00	0.99 $\pm$ 0.00	1.31 $\pm$ 0.10	1.72 $\pm$ 0.01
PBS	3	4	1.51 $\pm$ 0.14	1.36 $\pm$ 0.08	0.99 $\pm$ 0.00	1.00 $\pm$ 0.00	1.83 $\pm$ 0.06	1.78 $\pm$ 0.01

SUMMARY TABLES FOR MIXTURE ARRAY GENERATION EXPERIMENTS

Table S12. Results of array generation for mixtures in buffer with α -cyclodextrin

Jackknifed Classification Matrix

	Analytes 1 and 2	Analytes 1 and 3	Analytes 1 and 4	Analytes 2 and 3	Analytes 2 and 4	Analytes 3 and 4	%correct
Analytes 1 and 2	4	0	0	0	0	0	100
Analytes 1 and 3	0	4	0	0	0	0	100
Analytes 1 and 4	0	0	4	0	0	0	100
Analytes 2 and 3	0	0	0	4	0	0	100
Analytes 2 and 4	0	0	0	0	4	0	100
Analytes 3 and 4	0	0	0	0	0	4	100
Total	4	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.771	0.904	1.000
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Table S13. Results of array generation for mixtures in buffer with β -cyclodextrin

Jackknifed Classification Matrix

	Analytes 1 and 2	Analytes 1 and 3	Analytes 1 and 4	Analytes 2 and 3	Analytes 2 and 4	Analytes 3 and 4	%correct
Analytes 1 and 2	4	0	0	0	0	0	100
Analytes 1 and 3	0	4	0	0	0	0	100
Analytes 1 and 4	0	0	4	0	0	0	100
Analytes 2 and 3	0	0	0	4	0	0	100
Analytes 2 and 4	0	0	0	0	4	0	100
Analytes 3 and 4	0	0	0	0	0	4	100
Total	4	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.713	0.995	1.000
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Table S14. Results of array generation for mixtures in buffer with PBS

Jackknifed Classification Matrix

	Analytes 1 and 2	Analytes 1 and 3	Analytes 1 and 4	Analytes 2 and 3	Analytes 2 and 4	Analytes 3 and 4	%correct
Analytes 1 and 2	4	0	0	0	0	0	100
Analytes 1 and 3	0	4	0	0	0	0	100
Analytes 1 and 4	0	0	4	0	0	0	100
Analytes 2 and 3	0	0	0	4	0	0	100
Analytes 2 and 4	0	0	0	0	4	0	100
Analytes 3 and 4	0	0	0	0	0	4	100
Total	4	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.952	0.985	1.000
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Table S15. Results of array generation for mixtures in saliva with α -cyclodextrin

Jackknifed Classification Matrix

	Analytes 1 and 2	Analytes 1 and 3	Analytes 1 and 4	Analytes 2 and 3	Analytes 2 and 4	Analytes 3 and 4	%correct
Analytes 1 and 2	1	0	0	3	0	0	25
Analytes 1 and 3	0	4	0	0	0	0	100
Analytes 1 and 4	0	0	4	0	0	0	100
Analytes 2 and 3	1	0	0	3	0	0	75
Analytes 2 and 4	0	0	0	0	4	0	100
Analytes 3 and 4	0	0	0	0	0	4	100
Total	2	4	4	6	4	4	83

Cumulative Proportion of Total Dispersion

0.943	1.000	1.000
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Table S16. Results of array generation for mixtures in saliva with β -cyclodextrin

Jackknifed Classification Matrix

	Analytes 1 and 2	Analytes 1 and 3	Analytes 1 and 4	Analytes 2 and 3	Analytes 2 and 4	Analytes 3 and 4	%correct
Analytes 1 and 2	4	0	0	0	0	0	100
Analytes 1 and 3	0	4	0	0	0	0	100
Analytes 1 and 4	0	0	4	0	0	0	100
Analytes 2 and 3	0	0	0	4	0	0	100
Analytes 2 and 4	0	0	0	0	4	0	100
Analytes 3 and 4	0	0	0	0	0	4	100
Total	4	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.994	1.000	1.000
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Table S17. Results of array generation for mixtures in saliva with PBS

Jackknifed Classification Matrix

	Analytes 1 and 2	Analytes 1 and 3	Analytes 1 and 4	Analytes 2 and 3	Analytes 2 and 4	Analytes 3 and 4	%correct
Analytes 1 and 2	4	0	0	0	0	0	100
Analytes 1 and 3	0	4	0	0	0	0	100
Analytes 1 and 4	0	0	4	0	0	0	100
Analytes 2 and 3	0	0	0	4	0	0	100
Analytes 2 and 4	0	0	0	0	4	0	100
Analytes 3 and 4	0	0	0	0	0	4	100
Total	4	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.989	1.000	1.000
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SUMMARY FIGURES FOR FLUORESCENCE MODULATION EXPERIMENTS

Figure S6. Fluorescence modulation of fluorophore 6 with analyte 1 in buffer

Figure S7. Fluorescence modulation of fluorophore 7 with analyte 1 in buffer

Figure S8. Fluorescence modulation of fluorophore 8 with analyte 1 in buffer

Figure S9. Fluorescence modulation of fluorophore **6** with analyte **2** in buffer

Figure S10. Fluorescence modulation of fluorophore **7** with analyte **2** in buffer

Figure S11. Fluorescence modulation of fluorophore **8** with analyte **2** in buffer

Figure S12. Fluorescence modulation of fluorophore **6** with analyte **3** in buffer

Figure S13. Fluorescence modulation of fluorophore **7** with analyte **3** in buffer

Figure S14. Fluorescence modulation of fluorophore **8** with analyte **3** in buffer

Figure S15. Fluorescence modulation of fluorophore **6** with analyte **4** in buffer

Figure S16. Fluorescence modulation of fluorophore **7** with analyte **4** in buffer

Figure S17. Fluorescence modulation of fluorophore **8** with analyte **4** in buffer

Figure S18. Fluorescence modulation of fluorophore 6 with analyte 5 in buffer

Figure S19. Fluorescence modulation of fluorophore 7 with analyte 5 in buffer

Figure S20. Fluorescence modulation of fluorophore 8 with analyte 5 in buffer

Figure S21. Fluorescence modulation of fluorophore **6** with analyte **1** in saliva

Figure S22. Fluorescence modulation of fluorophore **7** with analyte **1** in saliva

Figure S23. Fluorescence modulation of fluorophore **8** with analyte **1** in saliva

Figure S24. Fluorescence modulation of fluorophore 6 with analyte 2 in saliva

Figure S25. Fluorescence modulation of fluorophore 7 with analyte 2 in saliva

Figure S26. Fluorescence modulation of fluorophore 8 with analyte 2 in saliva

Figure S27. Fluorescence modulation of fluorophore **6** with analyte **3** in saliva

Figure S28. Fluorescence modulation of fluorophore **7** with analyte **3** in saliva

Figure S29. Fluorescence modulation of fluorophore **8** with analyte **3** in saliva

Figure S30. Fluorescence modulation of fluorophore 6 with analyte 4 in saliva

Figure S31. Fluorescence modulation of fluorophore 7 with analyte 4 in saliva

Figure S32. Fluorescence modulation of fluorophore 8 with analyte 4 in saliva

Figure S33. Fluorescence modulation of fluorophore 6 with analyte 5 in saliva

Figure S34. Fluorescence modulation of fluorophore 7 with analyte 5 in saliva

Figure S35. Fluorescence modulation of fluorophore 8 with analyte 5 in saliva

SUMMARY FIGURES FOR LIMIT OF DETECTION EXPERIMENTS

Figure S36. Limit of detection for analyte **1** with fluorophore **6** and β -cyclodextrin in buffer

Figure S37. Limit of detection for analyte **2** with fluorophore **6** and β -cyclodextrin in buffer

Figure S38. Limit of detection for analyte **3** with fluorophore **6** and β -cyclodextrin in buffer

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CHAPTER 6

Published in *Analytical Methods* **2018**, 10, 3783-3790

Detection of Bisphenol A and Derivatives in Human Urine via Cyclodextrin-Promoted Fluorescence Modulation

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Manuscript 6

Detection of Bisphenol A and Derivatives in Human Urine via Cyclodextrin-Promoted Fluorescence Modulation

ABSTRACT

Reported herein is the sensitive and selective detection of bisphenol A (BPA) and six BPA derivatives in buffer and urine environments. This detection system relies on the ability of γ -cyclodextrin to act as a supramolecular scaffold to promote highly analyte-specific, proximity-induced fluorescence modulation of high quantum yield fluorophores, which led to unique modulation responses for each cyclodextrin-analyte-fluorophore combination investigated in both buffer and urine environments, and high selectivity between structurally similar analytes using linear discriminant analysis of the resulting response signals. This method was sensitive (low micromolar detection limits), selective (able to differentiate between structurally similar analytes), and broadly applicable (with successful detection in both buffer and urine environments), and has significant potential in the detection of BPA and its derivatives in a wide variety of complex environments.

INTRODUCTION

Among the list of known and suspected environmental toxicants, bisphenol A (BPA) and its derivatives,¹ including bisphenol S (BPS) and bisphenol F (BPF),² have received a significant amount of attention in both the scientific literature³ and the popular media. BPA is a suspected endocrine disruptor,⁴ and exposure to BPA and associated derivatives is correlated with increased rates of asthma,⁵ inflammatory bowel disease,⁶ endocrine disorders,⁷ and certain cancer sub-types.⁸ These negative health

effects are particularly concerning because of BPA's ubiquitous presence in a variety of commercial products, including plastic water bottles⁹ and bags,¹⁰ liners of canned food products,¹¹ infant bottles¹² and training (*i.e.* "sippy") cups,¹³ and cash register receipts.¹⁴ As a result of these deleterious health effects, many regulatory agencies have banned the use of BPA in commercial products.¹⁵ Manufacturers have replaced BPA with a variety of alternatives;¹⁶ however, the use of BPA-like derivatives are particularly concerning due to the limited amount of toxicity data associated with these compounds,¹⁷ and the fact that available data suggests that BPA derivatives can be even more toxic than BPA itself.¹⁸

There are almost no regulations associated with the use of BPA derivatives to date, nor are there any detailed regulations about the use of the label "BPA-free" on commercial products, which means that products that are labelled as "BPA-free" may contain significant amounts of BPA and BPA derivatives.¹⁹ One biological fluid that is particularly important for the detection of BPA and BPA derivatives is human urine, due to the non-invasive sample collection procedures and the relative simplicity of the matrix compared to other biological fluids.²⁰ BPA and its derivatives have been found in the urine of about 90% of the U.S. population.²¹ The relative dearth of regulations around BPA labelling,²² combined with the known and suspected deleterious health effects of BPA exposure,²³ and prevalence of BPA and its derivatives in human urine, means that methods for BPA detection that are sensitive, selective, rapid, and practical for real-world usage are sorely needed.

Reported methods for the detection of BPA and BPA derivatives focus predominantly on the use of mass spectrometry based methods, including gas

chromatography-mass spectrometry (GC-MS)²⁴ and high-pressure liquid chromatography (HPLC)²⁵ with mass spectrometry detection.²⁶ While these methods provide exquisite sensitivity for even trace amounts of BPA, their use comes with significant drawbacks in terms of the cost associated with such methods, the level of expertise required to operate this instrumentation, and the time required to achieve high levels of purification that enable such sensitive detection. Newer methods, including electrochemical assays,²⁷ colorimetric detection,²⁸ and Raman spectroscopy-based methods,²⁹ have also been reported.

Previous work in our group has focused on the detection of a variety of aromatic and non-aromatic toxicants, using cyclodextrin-promoted energy transfer³⁰ (for photophysically active toxicants) and cyclodextrin-promoted fluorescence modulation (for non-photophysically active analytes) (Fig. 1).³¹

Figure 1. Schematic of cyclodextrin-promoted (A) energy transfer and (B) fluorescence modulation

We demonstrated that this toxicant detection system operates in a broad range of environments, including plasma,³² breast milk,³³ and urine,³⁴ as well as in extracts

collected in the aftermath of the 2010 Deepwater Horizon oil spill.³⁵ Although our previous work detected polycyclic aromatic hydrocarbons,³⁶ polychlorinated biphenyls,³⁷ aromatic amines, pesticides,³⁸ aliphatic alcohols, and fuel spill components, we have not used cyclodextrin systems for the detection of BPA and BPA derivatives to date.

Reported herein is the rapid, sensitive, and selective detection of BPA and BPA derivatives in buffer and urine environments using cyclodextrin-promoted fluorescence modulation. This detection system uses commercially-available cyclodextrin hosts and fluorophore dyes to facilitate such detection. This method operates with high sensitivity (low micromolar detection limits), high selectivity (100% differentiation between structurally similar analytes), and general applicability (100% differentiation between binary mixtures of structurally similar analytes).

EXPERIMENTAL

Instruments and Reagents

All analytes and fluorophores (compounds **1-11**, Fig. 2) were purchased from Sigma-Aldrich Chemical Company and used as received, unless otherwise noted. γ -Cyclodextrin was purchased from Tokyo Chemical Industry and used as received. UV-Visible spectra were obtained using an Agilent 8453 UV/Visible spectrometer. Fluorescence spectra were obtained using a Shimadzu RF-5301PC spectrophotofluorimeter with 1.5 nm excitation and emission slit widths. All GC-MS measurements were obtained using a Shimadzu GC-MS QP2020 gas chromatograph-mass spectrometer. Urine was obtained from a single anonymous human donor and stored in amber high-density polyethylene bottles in the refrigerator until use.

Computational experiments were performed using Spartan 16 software. Arrays were generated using SYSTAT 13 statistical computing software.

Figure 2. Structures of analytes **1-7**, control analyte **8**, and fluorophores **9-11**

General Procedure for GC-MS Experiments

GC-MS sample preparation was conducted following literature-reported procedures.³⁹ Approximately 10 mL of the urine sample was added to a separatory funnel. Then, 15 mL of dichloromethane was added to the separatory funnel, and the separatory funnel was shaken vigorously. The organic layer was collected, and another 15 mL of dichloromethane was added to the urine layer. The organic layer was collected, another 15 mL of dichloromethane was added to the urine, and the procedure was repeated. The remaining organic layer was dried with MgSO_4 , filtered, concentrated to 1 mL using a rotary evaporator, and analysed by GC-MS.

All GC-MS measurements were obtained on a Shimadzu QP2020 gas

chromatograph-mass spectrometer with the following settings: column: Shimadzu SH-Rxi-5SilMS (30 m x 0.25 mm x 0.25 μ m); oven temperature: 45 $^{\circ}$ C, hold for 7 min, ramp to 200 $^{\circ}$ C at 20 $^{\circ}$ C/min, hold for 60 min; injection temperature: 200 $^{\circ}$ C; splitting ratio: splitless; MS ion source temperature: 230 $^{\circ}$ C; interface temperature: 150 $^{\circ}$ C; total run time: 75 min.

General Procedure for Fluorescence Modulation Experiments

In a quartz cuvette, 1.25 mL of a 10 mM γ -cyclodextrin solution dissolved in phosphate buffered saline (PBS) and 1.25 mL of a urine sample were combined and mixed thoroughly. For control experiments in the absence of urine, 2.5 mL of a γ -cyclodextrin solution was used. Next, 100 μ L of a 0.1 mg/mL fluorophore solution in tetrahydrofuran (THF) was added, and the solution was excited four times at the excitation wavelength of the fluorophore (460 nm for fluorophores **9** and **10** and 420 nm for fluorophore **11**). Then, 50 μ L of analytes **1-8** (1.0 mg/mL solution in THF) were added to the mixture, and the resulting solution was excited four times at the excitation wavelength of the fluorophore. The fluorescence emission spectra were integrated vs. wavenumber on the X-axis using OriginPro software, and the fluorescence modulation was measured by the ratio of the integrated emission of the fluorophore in the presence of the analyte to integrated emission of the fluorophore in the absence of the analyte, as shown in Equation 1:

$$\text{Fluorescence modulation} = Fl_{\text{analyte}}/Fl_{\text{blank}} \quad (\text{Eq. 1})$$

Where Fl_{analyte} is the integrated fluorescence emission of the fluorophore in the presence of analyte, and Fl_{blank} is the integrated fluorescence emission of the fluorophore in the absence of the analyte. Fluorescence modulation ratios greater than

1 indicate an enhancement of fluorescence emission of the fluorophore in the presence of analyte, fluorescence modulation ratios less than 1 indicate a decrease in fluorescence emission of the fluorophore in the presence of analyte, and fluorescence modulation ratios close to 1 indicate minimal change in fluorescence emission of the fluorophore in the presence of analyte.

General Procedure for Limit of Detection Calculations

The limit of detection (LOD) is defined as the lowest concentration of analyte at which a signal can be detected. To determine this value, the following steps were performed for each water-cyclodextrin-analyte combination.⁴⁰ In a quartz cuvette, 1.25 mL of 10 mM γ -cyclodextrin in PBS and 1.25 mL of urine sample were combined. In the absence of urine, 2.5 mL of γ -cyclodextrin solution was used. Then, 100 μ L of a fluorophore **9** (0.1 mg/mL solution in THF) was added, the solution was excited at 460 nm, and the fluorescence emission spectra were recorded. Six measurements were taken.

Next, 20 μ L of analyte (1.0 mg/mL in THF) were doped into an aqueous sample, and again the solution was excited at the fluorophore's excitation wavelength, and the fluorescence emission spectra were recorded. Six repeat measurements were taken. This step was repeated for 40 μ L of analyte, 60 μ L of analyte, 80 μ L of analyte, and 100 μ L of analyte, all of which were doped into a urine sample that did not initially contain toxicants, to measure the ability of the system to detect the doped toxicants within the complex aqueous matrix.

All of the fluorescence emission spectra were integrated vs. wavenumber on the X-axis using OriginPro software, and calibration curves were generated. The curves were plotted with the analyte concentration in μ M on the X-axis, and the fluorescence

modulation ratio on the Y-axis. The curve was fitted to a straight line and the equation of the line was determined (see ESI for full details). The limit of detection was calculated according to Equation 2:

$$\text{LOD} = 3(\text{SD}_{\text{blank}})/m \quad (\text{Eq. 2})$$

Where SD_{blank} is the standard deviation of the blank, analyte-free sample and m is the slope of the calibration curve. All LODs were reported in μM .

General Procedure for Array Generation Experiments

Array analysis was performed using SYSTAT 13 statistical computing software with the following settings: (a) Classical discriminant analysis; (b) Grouping variable: analytes; (c) Predictors: fluorophores; and (d) Long-range statistics: Mahal

General Procedure for Binding Constant Experiments

In a quartz cuvette, 2.5 mL of PBS and 50-100 μM of analyte were combined and mixed thoroughly. The absorbance of the sample was measured across the full UV and visible range. This procedure was repeated for $5.00 \times 10^{-6} \text{ M}$, $1.00 \times 10^{-5} \text{ M}$, $5.00 \times 10^{-5} \text{ M}$, $1.00 \times 10^{-4} \text{ M}$, $5.00 \times 10^{-4} \text{ M}$, $1.00 \times 10^{-3} \text{ M}$, $1.50 \times 10^{-3} \text{ M}$, and $2.50 \times 10^{-3} \text{ M}$ γ -cyclodextrin in PBS with constant analyte concentration (see ESI for full details). Double reciprocal Benesi-Hildebrand plots were generated following literature-reported procedures.⁴¹ Binding constants were calculated using Equation 3:

$$1/\Delta A = 1/b\Delta\epsilon[\text{G}][\text{H}]_0K_a + 1/b\Delta\epsilon[\text{H}]_0 \quad (\text{Eq. 3})$$

Where A is the change in absorbance, specifically the absorbance of analyte in the presence of cyclodextrin minus the absorbance of analyte in the blank, cyclodextrin-free sample in absorbance units, b is the path length of 1 cm, $\Delta\epsilon$ is the change in the absorption coefficient of the analyte-cyclodextrin complex minus the absorption

coefficient of the analyte in cyclodextrin-free solution in $\text{L}\cdot\text{cm}^{-1}\cdot\text{mol}^{-1}$, $[\text{G}]$ is the cyclodextrin concentration in molar (M), $[\text{H}]_0$ is the analyte concentration, and K_a is the binding constant in M^{-1} .

RESULTS AND DISCUSSION

Urine Characterization

GC-MS was used to characterize the analyte-free urine sample (Fig. 3).

Figure 3. GC-MS spectrum of human urine sample

The undoped urine sample showed peaks that corresponded to long-chain alkanes, fatty acids, and amides, all of which are commonly found in urine.⁴² Notably, the urine sample also contained GC-MS peaks that corresponded to benzophenone,⁴³ commonly used in personal care products such as sunscreen⁴⁴ and lip balm,⁴⁵ phthalates, commonly found in plastics⁴⁶ and some food products,⁴⁷ and acetaminophen, commonly found in over-the-counter pain relievers.⁴⁸

Selection of Fluorophores

Previous work in our group focused on the use of a BODIPY fluorophore with a free thiol moiety, which was synthesized in several steps. In order to develop more practically applicable detection systems, this work uses commercially available BODIPY compounds (fluorophores **9** and **10**), as well as coumarin 6 (fluorophore **11**),

which is commercially available, and has high quantum yields and excellent aqueous stability.

Selection of Cyclodextrins

Previous work in our group has used of a variety of commercially available cyclodextrins: α -cyclodextrin, β -cyclodextrin, randomly methylated β -cyclodextrin (with an average of 1.8 methyl groups per unit),⁴⁹ 2-hydroxypropyl- β -cyclodextrin, and γ -cyclodextrin, for cyclodextrin-promoted energy transfer and fluorescence modulation.

Due to the relatively large size of the analytes and the fluorophores in this system, γ -cyclodextrin was selected (Table 1 and Table 2). Of note, increases in the fluorophore emission intensity were observed in the presence of γ -cyclodextrin compared to in cyclodextrin-free solution, even in the absence of any added analyte (see ESI for more details). These increases can be attributed to the fact that binding in the cyclodextrin cavity decreases non-radiative decay pathways for the fluorophores and increases the resultant quantum yield, a phenomenon which has been observed previously.⁵⁰

Fluorescence Modulation

Fluorescence modulation experiments measure the ability of a particular analyte to induce measurable, analyte-specific changes in the fluorescence emission spectra of a high quantum yield fluorophore. Micromolar concentrations of analytes **1-7** or control analyte **8** were added to each cyclodextrin-fluorophore combination or urine-cyclodextrin-fluorophore combination. The degree of fluorescence modulation of fluorophores **9-11** in the presence of and absence of analyte was calculated using Eq. 1.

Based on computational results, it is evident that both the analyte and fluorophore do not bind in the cyclodextrin cavity together due to lack of available space inside the cavity (Table 1, Table 2), although the high flexibility of the cyclodextrin host means that shallow binding and/or deformation of the host in order to partially accommodate both guests cannot be ruled out.

Table 1. Calculated properties of analytes and fluorophores

Compound	Area ^a (Å ²)	Solvent-accessible surface area ^a (Å ²)	Volume ^a (Å ³)	Binding in γ - cyclodextrin in PBS ^b (M ⁻¹)	Electrostatic potential ^c (min/max) (kJ mol ⁻¹)
1	245	179	246	$3.22 \times 10^4 \pm 6.81 \times 10^3$	-141/273
2	259	184	265	$2.37 \times 10^4 \pm 2.72 \times 10^3$	-139/273
3	220	171	210	$1.39 \times 10^4 \pm 1.15 \times 10^4$	-139/273
4	233	179	225	$1.79 \times 10^4 \pm 1.98 \times 10^3$	-200/313
5	260	178	261	$7.83 \times 10^4 \pm 1.97 \times 10^3$	-122/295
6	295	214	307	$5.99 \times 10^4 \pm 3.83 \times 10^3$	-139/275
7	344	254	367	$1.06 \times 10^5 \pm 9.27 \times 10^2$	-140/275
8	104	84	86.3	^c	-139/68.2
9^d	262	194	266	$5.33 \times 10^3 \pm 1.47 \times 10^1$	-231/121
10^d	313	221	329	^c	-229/127
11^d	344	261	349	$1.75 \times 10^4 \pm 1.22 \times 10^2$	-202/118

^aValues were calculated using Spartan 16 software. ^bBinding constants calculated by UV/Visible spectroscopy (see ESI for more details). ^cNo binding constant value was obtained. ^dBinding constants measured by fluorescence spectroscopy.

Table 2. Literature-reported dimensions of γ -cyclodextrin^a

Compound	Outer diameter (Å)	Cavity diameter (Å)	Height of torus (Å)	Cavity volume (Å ³)
γ -cyclodextrin	17.5	7.5-8.3	7.9	427

^a Values were obtained from the literature⁵¹

Nonetheless, the addition of analyte presumably promotes non-covalent association of the fluorophore or other non-covalent interactions that lead to the observed fluorescence increases. It is important to note that while experimental conditions (i.e. temperature, pH, solvent, etc.) have an effect on the fluorescence modulation response in general,⁵² our system controls these particular experimental parameters both before and after analyte addition so that they remain consistent.

Specifically, in the case of fluorophore **9**, the fluorescence modulation values were greater than 1, indicating that introduction of analytes **1-7** and control analyte **8** led to an increase in the fluorescence emission of fluorophore **9** (Table 3). In this three-component system (analyte, fluorophore **9**, and cyclodextrin), the effect of analyte addition is to promote binding of the fluorophore inside the cyclodextrin cavity, resulting in the observed fluorescence increases.

Table 3. Fluorescence modulation values for analytes **1-8** with fluorophore **9**^a

Analyte	Buffer	Urine	Higher Ionic Strength Buffer
1	1.19 $\pm$ 0.01	1.36 $\pm$ 0.02	1.29 $\pm$ 0.02
2	1.20 $\pm$ 0.00	1.36 $\pm$ 0.02	^b
3	1.68 $\pm$ 0.01	1.85 $\pm$ 0.01	1.28 $\pm$ 0.01
4	1.19 $\pm$ 0.00	1.34 $\pm$ 0.01	^b
5	1.18 $\pm$ 0.00	1.38 $\pm$ 0.01	1.32 $\pm$ 0.03
6	1.19 $\pm$ 0.01	1.39 $\pm$ 0.01	^b
7	1.18 $\pm$ 0.01	1.36 $\pm$ 0.01	^b
8	1.17 $\pm$ 0.01	1.32 $\pm$ 0.00	1.29 $\pm$ 0.02

^aAll results represent an average of results from four trials for each sample. Fluorescence modulation values were calculated using Eq. 1. ^b Fluorescence modulation experiments were not performed.

Of note, the fluorescence modulation values in urine were uniformly higher than the values obtained in buffer, meaning that analyte addition in urine caused a larger increase in fluorescence compared to the fluorescence increases in buffer (see ESI for more details). Known differences in the composition of urine compared to buffer include higher concentrations of electrolytes as well as a lower pH value. For both factors, the net result is an increase in the concentration of charged species in solution, which can cause salt-induced increases in key intermolecular interactions, including hydrophobic association and intermolecular hydrogen bonding, following literature precedent.⁵³ As a control experiment, we also performed fluorescence modulation experiments using a higher ionic strength phosphate buffer with fluorophore **9** and selected analytes. In most cases, comparing the original PBS with the higher ionic strength PBS, resulted in greater increases in the fluorescence emission of fluorophore **9** in the presence of the higher ionic strength buffer. This supports the presence of salt-induced increases in intermolecular interactions. When comparing the fluorescence modulation in original PBS (conductivity = 11.6 $\mu\text{S}/\text{cm}$) with urine (conductivity = 16.38 $\mu\text{S}/\text{cm}$) with the higher ionic strength PBS (40.71 $\mu\text{S}/\text{cm}$), it was expected that as salt content increased, salt-induced intermolecular interactions would increase, leading to an increase in fluorescence modulation in original PBS, urine, and higher ionic strength PBS, respectively. These results indicate that the presence of other organic species in urine contribute to the higher degree of fluorescence modulation as compared to both purified buffer environments, and that the differences in results between buffer and urine are not solely due to the salt content. In comparing the fluorescence modulation values of different analytes in the original PBS, urine, and higher ionic

strength buffer, many of the analytes induce similar fluorescence changes regardless of the matrix, which again can be attributed to their ability to promote binding of the fluorophore **9** in the cyclodextrin cavity (Fig. 4).

Figure 4. Fluorescence changes of fluorophore **9** ($\lambda_{\text{ex}} = 460$ nm) in (A) buffer, (B) urine, and (C) higher ionic strength buffer with analytes **1**, **3**, **5**, and **8**. The black line represents analyte **1**, the red line represents analyte **3**, the blue line represents analyte **5**, and the purple line represents analyte **8**

Particularly high modulation values were observed for bisphenol F (analyte **3**), which is the only analyte that has no substitution at the methylene position. This structural anomaly, in turn, likely means that it has less steric hindrance and dimensions that enable it to bind in or associate close to the γ -cyclodextrin cavity with fluorophore **9**. (Table 1, Table 2). In contrast, the substitution of the methylene position with other analytes means that such ternary complex formation is less favored, and that other differences in substitution patterns (analyte **1** vs analyte **5**, for example) lead to minimal differences in the modulation values.

In most cases, the fluorescence modulation values for fluorophore **10** were less than 1, indicating that introduction of analytes **1-7** and control analyte **8** led to a decrease in the fluorescence emission of fluorophore **10** (Table 4). The responses for fluorophore **10** indicated the presence of a fluorophore excimer peak (Fig. 5), as a result of the binding of two molecules of fluorophore **10** in the γ -cyclodextrin cavity. In such cases,

introduction of the bisphenol analyte leads to the replacement of one of the two fluorophores from the cavity, leading to a decrease in the excimer emission and fluorescence modulation responses lower than 1.

Table 4. Fluorescence modulation values for analytes **1-8** with fluorophore **10**^a

Analyte	Buffer	Urine
1	1.03 ± 0.00	1.03 ± 0.00
2	0.86 ± 0.01	0.95 ± 0.00
3	0.68 ± 0.01	0.92 ± 0.01
4	0.84 ± 0.00	0.92 ± 0.01
5	0.83 ± 0.00	0.94 ± 0.01
6	0.84 ± 0.01	0.99 ± 0.01
7	0.81 ± 0.01	0.90 ± 0.00
8	1.03 ± 0.01	1.09 ± 0.01

^aAll results represent an average of results from four trials for each sample. Fluorescence modulation values were calculated using Eq. 1.

Figure 5. Fluorescence changes of fluorophore **10** ($\lambda_{\text{ex}} = 460$ nm) in (A) buffer and (B) urine with analytes **7** and **8**. The black line represents no analyte, the red line represents analyte **7**, and the blue line represents analyte **8**

In every case, the fluorescence modulation values for fluorophore **11** were greater than 1, indicating that introduction of analytes **1-7** and control analyte **8** led to an increase in the fluorescence emission of fluorophore **11** (Table 5). These increases were generally lower in the magnitude that the increases observed for fluorophore **9**, indicating that the analyte-induced changes in the binding of fluorophore **11** in the

cavity were lower than those of fluorophores **9** and **10**. Additionally, the signal-to-noise ratio of all spectra containing fluorophore **11** is lower than spectra obtained in the presence of fluorophores **9** and **10**. This different behaviour is likely a result of the fact that fluorophores **9** and **10** have higher quantum yields compared to fluorophore **11**,⁵⁴ and that as a result of both of these features, binding of fluorophore **11** in the γ -cyclodextrin cavity is more highly favoured. In support of this statement, electrostatic potential mapping of fluorophore **11** and fluorophores **9** and **10** show marked differences, both in terms of variation in electrostatic potential as well as in overall size and shape of the fluorophore (see ESI for more details). These marked differences in the electrostatic potentials as well as solvent accessible surface areas (Table 1) contribute to the differences in interactions for each cyclodextrin-fluorophore-analyte combination.

Table 5. Fluorescence modulation values for analytes **1-8** with fluorophore **11**^a

Analyte	Buffer	Urine
1	1.06 $\pm$ 0.00	1.08 $\pm$ 0.00
2	1.08 $\pm$ 0.01	1.08 $\pm$ 0.01
3	1.07 $\pm$ 0.01	1.08 $\pm$ 0.00
4	1.07 $\pm$ 0.002	1.08 $\pm$ 0.00
5	1.07 $\pm$ 0.003	1.07 $\pm$ 0.00
6	1.07 $\pm$ 0.003	1.05 $\pm$ 0.01
7	1.09 $\pm$ 0.004	1.08 $\pm$ 0.01
8	1.09 $\pm$ 0.01	1.08 $\pm$ 0.00

^aAll results represent an average of results from four trials for each sample. Fluorescence modulation values were calculated using Eq. 1.

Fluorophore **11** has a number of advantages in terms of detection applications (high quantum yield, aqueous stability, etc), and our group has used it previously for a variety of turn-on detection schemes. The differential performance of fluorophores **9**

and **10** compared to fluorophore **11** in this context provides important information for the array-based selectivity.

Array Generation

The selectivity of this system for structurally similar bisphenol derivatives was determined using array-based analysis of the fluorescence response patterns. This analysis resulted in 100% differentiation between analytes in both purified buffer and urine (Fig. 6A and 6C). The response patterns are markedly distinct for buffer and urine environments, and show unique, well-separated signals for structurally similar bisphenols within each sample. The high degree of success and noticeable visual differences between environments highlight the power of this statistical method in distinguishing even very slight structural differences (analytes **1** and **2**, analytes **1** and **5**, and analytes **6** and **7**). Given the high degree of fluorescence modulation of fluorophores **9** and **10** and slight change of fluorescence of fluorophore **11**, arrays were also generated using only data from fluorophores **9** and **10** (Fig. 6B and 6D).

Interestingly, these arrays led to 88% differentiation between analytes. These arrays are visually different than the arrays generated using fluorophores **9-11** as predictors. There is more clustering between analytes, indicating poor separation between structurally similar analytes. This indicates that although the changes in fluorescence emission of fluorophore **11** upon analyte addition are minimal, they add more information and enable effective separation between structurally similar analytes.

Figure 6. Array-based detection of analytes **1-8** in (A) buffer with fluorophores **9-11** as predictors; (B) buffer with fluorophores **9** and **10** as predictors; (C) urine with fluorophores **9-11** as predictors; and (D) urine with fluorophores **9** and **10** as predictors

In complex samples, such as human urine, it is likely multiple bisphenol analogues will be present in the event of an exposure event, or from cumulative exposure to bisphenol-containing consumer products. To address this issue, we tested the ability of our array-based detection system to differentiate between binary mixtures of bisphenol analytes (Fig. 7). Interestingly, analyte mixtures grouped on one side of the array, and samples containing one single analyte grouped on the other side of the array. This array corresponded to 100% differentiation between analyte mixtures and single analytes. Current work in our laboratory is focused on expanding these studies to

include ternary and quaternary mixtures of analytes as well as different ratios of analytes in each mixture.

Figure 7. Array-based detection of binary mixtures of analytes in urine using fluorophores **9-11** as predictors

Limit of Detection

In addition to the ability to selectively differentiate between structurally similar analytes, it is important to be able to detect these compounds at low levels. The sensitivity of the system was determined by calculating limits of detection for all samples with fluorophore **9** following literature-reported procedures. Fluorophore **9** was chosen for these experiments because interactions with fluorophore **9** led to the highest degree of fluorescence modulation therefore indicating extremely sensitive detection. The results of these studies are summarized in Table 6.

In general, the limits of detection for analytes in urine were slightly higher than those measured in purified buffer solutions. This is likely a result of the higher innate levels of nonpolar molecules that may compete with analytes for binding in the cyclodextrin cavity. These interactions can interfere with favourable intermolecular

interactions and slightly lower the system sensitivity. While our low part-per-million range detection limits show promise for the use of this detection system in other complex environments, the concentration of bisphenol compounds found in urine is in the part-per-billion range.⁵⁵ Current efforts in our laboratory are focused on lowering detection limits for cases where the detection limits are above the literature-reported exposure limits by looking at other cyclodextrin-fluorophore combinations, and/or employing a preconcentration step to achieve optimal sensitivities.

Table 6. LODs for analytes **1-7** with fluorophore **9** in buffer and urine^a

Analyte	LOD in Buffer (ppm)	LOD in Urine (ppm)
1	1.51 ± 0.05	1.82 ± 0.03
2	0.14 ± 0.00	2.30 ± 0.03
3	0.01 ± 0.00	0.40 ± 0.01
4	0.29 ± 0.00	0.57 ± 0.00
5	0.39 ± 0.01	0.84 ± 0.01
6	0.49 ± 0.02	2.80 ± 0.04
7	0.39 ± 0.01	0.28 ± 0.01

^aLODs were calculated using the procedures.⁴⁰ See Electronic Supporting Information for full details.

CONCLUSIONS

In summary, cyclodextrin-promoted fluorescence modulation can be used for the detection of BPA and its derivatives in both buffer and urine environments. This method is sensitive (low micromolar detection limits), selective (able to differentiate between structurally similar analytes), and broadly applicable (success in detecting analytes in buffer and complex urine environments). Future work in our laboratory is focused on translating this highly sensitive and selective bisphenol detection method into a practical, solid-state detection device for the facile, at-home detection of BPA and derivatives in contaminated aqueous samples.

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Detection of Bisphenol A and Derivatives in Human Urine via Cyclodextrin-

Promoted Fluorescence Modulation

ANALYTE DETAILS

Figure S1. Structures of analytes 1-7, control analyte 8, and fluorophores 9-11**Table S1.** Final solution concentrations of analytes and fluorophores

Compound	Volume Added (μL)	Final Concentration (μM)
1	50	84.24
2	50	79.36
3	50	96.04
4	50	76.84
5	50	57.19
6	50	66.23
7	50	54.57
8	50	266.7
9	100	14.67
10	100	10.11
11	100	10.98

SUMMARY TABLES FOR FLUORESCENCE MODULATION EXPERIMENTS

Table S2. Fluorescence modulation results for each analyte in buffer

Analyte	Fluorophore	Fluorescence Modulation
1	9	1.19 ± 0.01
	10	1.03 ± 0.002
	11	1.06 ± 0.004
2	9	1.20 ± 0.001
	10	0.86 ± 0.01
	11	1.08 ± 0.01
3	9	1.68 ± 0.01
	10	0.68 ± 0.01
	11	1.07 ± 0.01
4	9	1.19 ± 0.004
	10	0.84 ± 0.003
	11	1.07 ± 0.002
5	9	1.18 ± 0.002
	10	0.83 ± 0.004
	11	1.07 ± 0.003
6	9	1.19 ± 0.01
	10	0.84 ± 0.01
	11	1.07 ± 0.003
7	9	1.18 ± 0.01
	10	0.81 ± 0.01
	11	1.09 ± 0.004
8	9	1.17 ± 0.01
	10	1.03 ± 0.01
	11	1.09 ± 0.01

Table S3. Fluorescence modulation results for each analyte in urine

Analyte	Fluorophore	Fluorescence Modulation
1	9	1.36 ± 0.02
	10	1.03 ± 0.002
	11	1.08 ± 0.004
2	9	1.36 ± 0.02
	10	0.95 ± 0.001
	11	1.08 ± 0.01
3	9	1.85 ± 0.01
	10	0.92 ± 0.01
	11	1.08 ± 0.004
4	9	1.34 ± 0.01
	10	0.92 ± 0.01
	11	1.08 ± 0.004
5	9	1.38 ± 0.01
	10	0.94 ± 0.01
	11	1.07 ± 0.004
6	9	1.39 ± 0.01
	10	0.99 ± 0.01
	11	1.05 ± 0.01
7	9	1.36 ± 0.01
	10	0.90 ± 0.004
	11	1.08 ± 0.01
8	9	1.32 ± 0.003
	10	1.09 ± 0.01
	11	1.08 ± 0.004

SUMMARY TABLES FOR LIMIT OF DETECTION EXPERIMENTS

Table S4. Limits of detection for analytes with fluorophore **9** in buffer

Analyte	Fluorophore	Equation	R ²	LOD (μM)
1	9	$y = 0.0026x + 1.0307$	0.9998	6.61 ± 0.21
2	9	$y = 0.0027x + 1.0177$	0.99763	0.57 ± 0.01
3	9	$y = 0.007x + 0.8928$	0.99774	0.049 ± 0.00
4	9	$y = 0.0032x + 1.0078$	0.99905	1.16 ± 0.00
5	9	$y = 0.0037x + 1.0299$	0.99671	1.18 ± 0.02
6	9	$y = 0.0037x + 0.9999$	0.99822	1.70 ± 0.06
7	9	$y = 0.0044x + 0.9972$	0.99802	1.11 ± 0.03

Table S5. Limits of detection for analytes with fluorophore **9** in urine

Analyte	Fluorophore	Equation	R ²	LOD (μM)
1	9	$y = 0.0043x + 1.0826$	0.99573	7.96 ± 0.11
2	9	$y = 0.0044x + 0.9991$	0.99798	9.50 ± 0.13
3	9	$y = 0.009x + 0.9322$	0.99197	2.01 ± 0.06
4	9	$y = 0.0042x + 0.9673$	0.9969	2.29 ± 0.00
5	9	$y = 0.0049x + 1.0074$	0.99773	2.50 ± 0.03
6	9	$y = 0.0042x + 1.016$	0.99108	9.66 ± 0.13
7	9	$y = 0.0043x + 1.0434$	0.9815	0.79 ± 0.02

SUMMARY TABLES FOR ARRAY GENERATION EXPERIMENTS

Table S6. Results of array generation in buffer

Jackknifed Classification Matrix

	Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	Analyte 6	Analyte 7	Analyte 8	%correct
Analyte 1	4	0	0	0	0	0	0	0	100
Analyte 2	0	4	0	0	0	0	0	0	100
Analyte 3	0	0	4	0	0	0	0	0	100
Analyte 4	0	0	0	4	0	0	0	0	100
Analyte 5	0	0	0	0	4	0	0	0	100
Analyte 6	0	0	0	0	0	4	0	0	100
Analyte 7	0	0	0	0	0	0	4	0	100
Analyte 8	0	0	0	0	0	0	0	4	100
Total	4	4	4	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.925	0.988	1.000
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Table S7. Results of array generation in urine

Jackknifed Classification Matrix

	Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	Analyte 6	Analyte 7	Analyte 8	%correct
Analyte 1	4	0	0	0	0	0	0	0	100
Analyte 2	0	4	0	0	0	0	0	0	100
Analyte 3	0	0	4	0	0	0	0	0	100
Analyte 4	0	0	0	4	0	0	0	0	100
Analyte 5	0	0	0	0	4	0	0	0	100
Analyte 6	0	0	0	0	0	4	0	0	100
Analyte 7	0	0	0	0	0	0	4	0	100
Analyte 8	0	0	0	0	0	0	0	4	100
Total	4	4	4	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.810	0.994	1.000
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SUMMARY TABLES FOR GC-MS CHARACTERIZATION

Table S8. GC-MS results for urine

Time (min)	NIST Compound ID
10	6-methyl-3-cyclohexen-1-carboxaldehyde
10.25	2,4,4-Trimethyl-1-hexene
12	2-Methylbenzoxazol
12.4	Dehydromevalonic lactone
12.5	1-methyl-2-piperidinone
12.6	Dodecane
12.8	Mannosamine
13.1	1-Butyrylpyrrolidine
13.25	Carbonic acid, eicosyl prop-e-en-2-yl ester
13.3	2-Isopopyl-5-methyl-1-hexanol
13.45	11-Methyldodecanol
13.5	1,4-Diethylhexyl dichloroacetate
13.55	Dodecyl nonyl ether
13.65	2,4-Dimethylheptane
13.7	2,3,5-Trimethyldecane
13.75	3,8-Dimethyldecane
13.85	Tetradecane
13.95	Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester
15	Hexadecane
15.1	Acetaminophen
15.2	Cyclohexyl laurate
15.4	Benzophenone
15.6	2-hexyldecanol
16	3,7,11,15-Tetramethyl-2-hexadecene
16.15	2-Methylhexacosane
16.25	1-(15-Methylhexadecanoyl)pyrrolidine
18.3	Bis(tridecyl) phthalate
18.4	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine
18.5	Dodecanamide
22.8	Hexadecanamide
29.5	9-Octadecenamide
44	Diisooctyl phthalate

SUMMARY TABLE FOR MIXTURE FLUORESCENCE MODULATION EXPERIMENTS

Table S9. Fluorescence modulation results for analyte mixtures in urine

Analyte	Analyte	Fluorophore 9	Fluorophore 10	Fluorophore 11
1	2	1.06 ± 0.05	1.11 ± 0.004	1.07 ± 0.002
1	3	1.12 ± 0.05	1.10 ± 0.004	1.09 ± 0.0003
1	4	1.10 ± 0.03	1.09 ± 0.01	1.06 ± 0.003
1	5	1.02 ± 0.08	1.09 ± 0.01	1.07 ± 0.002
1	6	1.07 ± 0.02	1.10 ± 0.001	1.05 ± 0.003
1	7	1.03 ± 0.05	1.07 ± 0.002	1.05 ± 0.004
2	3	1.00 ± 0.09	1.12 ± 0.002	1.08 ± 0.001
3	4	1.07 ± 0.07	1.09 ± 0.01	1.08 ± 0.004
3	5	1.03 ± 0.08	1.10 ± 0.01	1.08 ± 0.01
6	7	0.99 ± 0.07	1.08 ± 0.002	1.06 ± 0.003

SUMMARY TABLE FOR MIXTURE ARRAY GENERATION EXPERIMENTS

Table S10. Results of array generation for analytes mixtures in urine

Jackknifed Classification Matrix

	Analytes 1 and 2	Analytes 1 and 3	Analytes 1 and 4	Analytes 1 and 5	Analytes 1 and 6	Analytes 1 and 7	Analytes 2 and 3
Analytes 1 and 2	4	0	0	0	0	0	0
Analytes 1 and 3	0	4	0	0	0	0	0
Analytes 1 and 4	0	0	4	0	0	0	0
Analytes 1 and 5	0	0	0	4	0	0	0
Analytes 1 and 6	0	0	0	0	4	0	0
Analytes 1 and 7	0	0	0	0	0	4	0
Analytes 2 and 3	0	0	0	0	0	0	4
Analytes 3 and 4	0	0	0	0	0	0	0
Analytes 3 and 5	0	0	0	0	0	0	0
Analytes 6 and 7	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	Analytes 3 and 4	Analytes 3 and 5	Analytes 6 and 7	%correct
Analytes 1 and 2	0	0	0	100
Analytes 1 and 3	0	0	0	100
Analytes 1 and 4	0	0	0	100
Analytes 1 and 5	0	0	0	100
Analytes 1 and 6	0	0	0	100
Analytes 1 and 7	0	0	0	100
Analytes 2 and 3	0	0	0	100
Analytes 3 and 4	4	0	0	100
Analytes 3 and 5	0	4	0	100
Analytes 6 and 7	0	0	4	100
Total	4	4	4	100

Cumulative Proportion of Total Dispersion

0.766	0.998	1.000
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SUMMARY TABLES FOR BINDING CONSTANT EXPERIMENTS

Table S11. UV/Visible spectroscopy binding constants for analytes **1-7** with γ -cyclodextrin

Analyte	Concentration (M)	λ_{max} (nm)	Equation	R ²	K _a (M ⁻¹)
1	1.01 x 10 ⁻⁴	284	y = 0.0005x + 11.906	0.9586	3.22 x 10 ⁴ ± 6.81 x 10 ³
2	1.02 x 10 ⁻⁴	285	y = 0.0005x + 11.473	0.9595	2.37 x 10 ⁴ ± 2.72 x 10 ³
3	5.18 x 10 ⁻⁵	286	y = 0.0003x + 7.7833	0.9861	1.39 x 10 ⁴ ± 1.15 x 10 ⁴
4	5.07 x 10 ⁻⁵	297	y = 0.0005x + 9.9003	0.901	1.79 x 10 ⁴ ± 1.98 x 10 ³
5	5.15 x 10 ⁻⁵	280	y = 0.0001 + 11.405	0.9471	7.83 x 10 ⁴ ± 1.97 x 10 ³
6	5.30 x 10 ⁻⁵	287	y = 0.0002x + 9.1613	0.9414	5.99 x 10 ⁴ ± 3.83 x 10 ³
7	5.02 x 10 ⁻⁶	250	y = 0.0003x + 3.4255	0.9553	1.06 x 10 ⁵ ± 9.27 x 10 ²

SUMMARY FIGURES FOR FLUORESCENCE MODULATION EXPERIMENTS

Figure S2. Fluorescence modulation of analyte 1 in buffer

Figure S3. Fluorescence modulation of analyte 2 in buffer

Figure S4. Fluorescence modulation of analyte 3 in buffer

Figure S5. Fluorescence modulation of analyte 4 in buffer

Figure S6. Fluorescence modulation of analyte 5 in buffer

Figure S7. Fluorescence modulation of analyte 6 in buffer

Figure S8. Fluorescence modulation of analyte 7 in buffer

Figure S9. Fluorescence modulation of control analyte 8 in buffer

Figure S10. Fluorescence modulation of analyte 1 in urine

Figure S11. Fluorescence modulation of analyte 2 in urine

Figure S12. Fluorescence modulation of analyte 3 in urine

Figure S13. Fluorescence modulation of analyte 4 in urine

Figure S14. Fluorescence modulation of analyte 5 in urine

Figure S15. Fluorescence modulation of analyte 6 in urine

Figure S16. Fluorescence modulation of analyte 7 in urine

Figure S17. Fluorescence modulation of control analyte **8** in urine

SUMMARY FIGURES FOR LIMIT OF DETECTION EXPERIMENTS

Figure S18. Limit of detection for analyte **1** in buffer

Figure S19. Limit of detection for analyte **2** in buffer

Figure S20. Limit of detection for analyte **3** in buffer

Figure S21. Limit of detection for analyte **4** in buffer

Figure S22. Limit of detection for analyte **5** in buffer

Figure S23. Limit of detection for analyte **6** in buffer

Figure S24. Limit of detection for analyte **7** in buffer

Figure S25. Limit of detection for analyte **1** in urine

Figure S26. Limit of detection for analyte **2** in urine

Figure S27. Limit of detection for analyte **3** in urine

Figure S28. Limit of detection for analyte **4** in urine

Figure S29. Limit of detection for analyte **5** in urine

Figure S30. Limit of detection for analyte **6** in urine

Figure S31. Limit of detection for analyte **7** in urine

SUMMARY FIGURES FOR ARRAY GENERATION EXPERIMENTS

Figure S32. Linear discriminant analysis of fluorescence responses for analytes **1-8** with fluorophores **9-11** in buffer

Figure S33. Linear discriminant analysis of fluorescence responses for analytes **1-8** with fluorophores **9-11** in urine

SUMMARY FIGURES FOR GC-MS CHARACTERIZATION

Figure S34. GC-MS trace for urine for undoped sample at 10 minutes

Figure S35. GC-MS trace for urine for undoped sample at 10.25 minutes

Figure S36. GC-MS trace for urine for undoped sample at 12 minutes

Figure S37. GC-MS trace for urine for undoped sample at 12.4 minutes

Figure S38. GC-MS trace for urine for undoped sample at 12.5 minutes

Figure S39. GC-MS trace for urine for undoped sample at 12.6 minutes

Figure S40. GC-MS trace for urine for undoped sample at 12.8 minutes

Figure S41. GC-MS trace for urine for undoped sample at 13.1 minutes

Figure S42. GC-MS trace for urine for undoped sample at 13.25 minutes

Figure S43. GC-MS trace for urine for undoped sample at 13.3 minutes

Figure S44. GC-MS trace for urine for undoped sample at 13.45 minutes

Figure S45. GC-MS trace for urine for undoped sample at 13.5 minutes

Figure S48. GC-MS trace for urine for undoped sample at 13.7 minutes

Figure S49. GC-MS trace for urine for undoped sample at 13.75 minutes

Figure S50. GC-MS trace for urine for undoped sample at 13.85 minutes

Figure S51. GC-MS trace for urine for undoped sample at 13.95 minutes

Figure S52. GC-MS trace for urine for undoped sample at 15 minutes

Figure S53. GC-MS trace for urine for undoped sample at 15.1 minutes

Figure S54. GC-MS trace for urine for undoped sample at 15.2 minutes

Figure S55. GC-MS trace for urine for undoped sample at 15.4 minutes

Figure S56. GC-MS trace for urine for undoped sample at 15.6 minutes

Figure S57. GC-MS trace for urine for undoped sample at 16 minutes

Figure S58. GC-MS trace for urine for undoped sample at 16.15 minutes

Figure S59. GC-MS trace for urine for undoped sample at 16.25 minutes

Figure S60. GC-MS trace for urine for undoped sample at 18.3 minutes

Figure S61. GC-MS trace for urine for undoped sample at 18.4 minutes

Figure S62. GC-MS trace for urine for undoped sample at 18.5 minutes

Figure S63. GC-MS trace for urine for undoped sample at 22.8 minutes

Figure S64. GC-MS trace for urine for undoped sample at 29.5 minutes

Figure S65. GC-MS trace for urine for undoped sample at 44 minutes

SUMMARY FIGURES FOR MIXTURE FLUORESCENCE MODULATION EXPERIMENTS

Figure S66. Fluorescence modulation of fluorophores 9-11 with analytes 1 and 2 in urine

Figure S67. Fluorescence modulation of fluorophores 9-11 with analytes 1 and 3 in urine

Figure S68. Fluorescence modulation of fluorophores 9-11 with analytes 1 and 4 in urine

Figure S69. Fluorescence modulation of fluorophores **9-11** with analytes **1** and **5** in urine

Figure S70. Fluorescence modulation of fluorophores **9-11** with analytes **1** and **6** in urine

Figure S71. Fluorescence modulation of fluorophores **9-11** with analytes **1** and **7** in urine

Figure S72. Fluorescence modulation of fluorophores **9-11** with analytes **2** and **3** in urine

Figure S73. Fluorescence modulation of fluorophores **9-11** with analytes **3** and **4** in urine

Figure S74. Fluorescence modulation of fluorophores **9-11** with analytes **3** and **5** in urine

Figure S75. Fluorescence modulation of fluorophores **9-11** with analytes **6** and **7** in urine

SUMMARY FIGURES FOR MIXTURE ARRAY GENERATION EXPERIMENTS

Figure S76. Linear discriminant analysis of fluorescence responses for analyte mixtures with fluorophores **9-11** in urine

SUMMARY FIGURES FOR ELECTROSTATIC POTENTIAL MAPPING

Figure S77. Electrostatic potential map of analyte 1

Figure S78. Electrostatic potential map of analyte 2

Figure S79. Electrostatic potential map of analyte 3

Figure S80. Electrostatic potential map of analyte 4

Figure S81. Electrostatic potential map of analyte 5

Figure S82. Electrostatic potential map of analyte 6

Figure S83. Electrostatic potential map of analyte 7

Figure S84. Electrostatic potential map of analyte 8

Figure S85. Electrostatic potential map of fluorophore 9

Figure S86. Electrostatic potential map of fluorophore **10**

Figure S87. Electrostatic potential map of fluorophore **11**

**SUMMARY FIGURES FOR FLUOROPHORES 9-11 FLUORESCENCE
EMISSION SPECTRA**

Figure S88. Fluorescence emission of fluorophore **9** in the absence and presence of cyclodextrin in buffer

Figure S89. Fluorescence emission of fluorophore **10** in the absence and presence of cyclodextrin in buffer

Figure S90. Fluorescence emission of fluorophore **11** in the absence and presence of cyclodextrin in buffer

Figure S91. Fluorescence emission of fluorophore **9** in the absence and presence of cyclodextrin in urine

Figure S92. Fluorescence emission of fluorophore **10** in the absence and presence of cyclodextrin in urine

Figure S93. Fluorescence emission of fluorophore **11** in the absence and presence of cyclodextrin in urine

SUMMARY FIGURES FOR BINDING CONSTANT EXPERIMENTS

Figure S94. Double reciprocal Benesi-Hildebrand plot for analyte **1** with γ -cyclodextrin

Figure S95. Double reciprocal Benesi-Hildebrand plot for analyte **2** with γ -cyclodextrin

Figure S96. Double reciprocal Benesi-Hildebrand plot for analyte **3** with γ -cyclodextrin

Figure S97. Double reciprocal Benesi-Hildebrand plot for analyte **4** with γ -cyclodextrin

Figure S98. Double reciprocal Benesi-Hildebrand plot for analyte **5** with γ -cyclodextrin

Figure S99. Double reciprocal Benesi-Hildebrand plot for analyte **6** with γ -cyclodextrin

Figure S100. Double reciprocal Benesi-Hildebrand plot for analyte **7** with γ -cyclodextrin