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TOXICANT ISOMERS CHARACTERIZED USING
FLUORESCENCE EMISSION OF COUMARIN ATTACHED
TO BETA-CYCLODEXTRIN

BY

BENJAMIN R. SMITH

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
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IN
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OF
BENJAMIN R. SMITH

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2017

ABSTRACT

Chapter 1 reports a highly sensitive and selective array-based sensing strategy for classifying isomeric and analogous analytes based on their differential interactions with three supramolecular cyclodextrin-fluorophore sensors. Each analyte-sensor interaction results in a distinct fluorescence modulation response, and these variable responses are then statistically classified via linear discriminant analyses (LDA) into clusters of maximum separation. Three classes of isomeric analytes (aromatic alcohols, aliphatic alcohols, and hexanes) and two classes of analogous analytes (analogues of dichlorodiphenyltrichloroethane (DDT) and congeners of polychlorinated biphenyls (PCBs)) have been successfully classified with 100% accuracy. High sensitivity of this sensor is demonstrated as well, with limits of detection approaching or surpassing known levels of concern, and preliminary efforts at successfully classifying binary analyte mixtures using this sensor system are also reported.

Chapter 2 discusses the extensive literature reported on the properties of pyrene in β -cyclodextrin and γ -cyclodextrin. Despite this literature, little has been published on the interactions of pyrene with β -cyclodextrin derivatives (methyl- β -cyclodextrin and 2-hydroxypropyl- β -cyclodextrin) or mixtures of different cyclodextrins. These experiments focused on characterizing pyrene's interactions, as well as those of perdeuterated pyrene-d10 and benzo[*a*]pyrene, within different cyclodextrin solutions using fluorescence spectroscopy. The vibronic bands within the fluorescence emission spectrum of pyrene reflected the polarity of the

microenvironment around pyrene (characterized by Py values). Little change in the Py values was observed when pyrene was introduced to different concentrations of methyl- β -cyclodextrin and 2-hydroxypropyl- β -cyclodextrin. Benzo[*a*]pyrene had a large excimer peak when doped into γ - or β : γ solutions, which suggested that benzo[*a*]pyrene may be forming 2:1 complexes with the cyclodextrins. Deuterated pyrene (pyrene-d10) had a lower binding constant in β -cyclodextrin (compared to pyrene-h10), but a higher binding constant in γ -cyclodextrin (compared to pyrene-h10). Further studies should be conducted to determine why deuterium incorporation would produce a higher binding constant in the γ -cyclodextrin solutions.

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PREFACE

This dissertation is presented in manuscript format according to the guidelines of the graduate school of the University of Rhode Island. Two manuscripts will be presented in this thesis. Chapter 1 is submitted for publication to *Sensors and Actuators B: Chemical* with the authors Sauradip Chaudhuri, Dana J. DiScenza, Benjamin Smith, Reid Yocum, and Mindy Levine. Chapter 2 is being prepared for submission to *Supramolecular Chemistry* with the authors Benjamin Smith and Mindy Levine.

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CHAPTER 1

Submitted for publication to *Sensors and Actuators B: Chemical*

Array-based detection of isomeric and analogous analytes employing synthetically
modified fluorophore attached β -cyclodextrin derivatives

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Manuscript 1

Array-based detection of isomeric and analogous analytes employing synthetically modified fluorophore attached β -cyclodextrin derivatives

ABSTRACT

Reported herein is a highly sensitive and selective array-based sensing strategy for classifying isomeric and analogous analytes based on their differential interactions with three supramolecular cyclodextrin-fluorophore sensors. Each analyte-sensor interaction results in a distinct fluorescence modulation response, and these variable responses are then statistically classified via linear discriminant analyses (LDA) into clusters of maximum separation. Three classes of isomeric analytes (aromatic alcohols, aliphatic alcohols, and hexanes) and two classes of analogous analytes (analogues of dichlorodiphenyltrichloroethane (DDT) and analogues of polychlorinated biphenyls (PCBs)) have been successfully classified with 100% accuracy. High sensitivity of this sensor is demonstrated as well, with limits of detection approaching or surpassing known levels of concern, and preliminary efforts at successfully classifying binary analyte mixtures using this sensor system are also reported.

INTRODUCTION

The selective detection and accurate quantification of structurally similar analytes is a major environmental challenge for chemists and toxicologists, as structurally similar analytes often have widely disparate toxicities and environmental degradation pathways.¹ The most common strategy to address

this challenge is to use mass spectrometry based methods such as liquid chromatography-mass spectrometry (LC-MS)² or gas chromatography-mass spectrometry (GC-MS).³ However, there are significant drawbacks associated with this approach, including the significant costs and time necessary to conduct such analyses,⁴ which limits the ability to conduct high throughput assays.⁵

An alternate strategy is to use array-based sensing systems, which have gained in popularity in recent years.⁶ This approach relies on the development of a chemical signature for each analyte based on analyte-specific interactions with a series of sensors. In a multi-component system, each individual analyte develops a unique response pattern, which is then compared against known samples to enable accurate identification.

Array-based sensing systems can be combined with supramolecular sensors, which rely on differential non-covalent interactions of isomeric analytes with supramolecular hosts, including cyclodextrins,⁷ fluorescent polymers,⁸ molecularly imprinted polymers,⁹ and metal-organic frameworks (MOFs).¹⁰ Cyclodextrin-based detection systems in particular have used either covalent¹¹ or non-covalent attachment¹² of a spectroscopically active unit to achieve a read-out signal. However, often the detection specificity in this kind of sensing is limited due to structural similarities among related groups of analytes.

Although supramolecular array-based systems overcome many challenges associated with mass-spectrometry based detection methods, the analyte scope explored in most of these reports have been limited to aromatic

small molecules such as xylenes¹³ and nitrotoluenes.¹⁴ In a real-world contaminated environment, the nature of the various pollutants is highly complex,¹⁵ and includes complex mixtures of aromatic and non-aromatic compounds.¹⁶ This kind of situation requires the development of a sensing system which is rapid, simple and efficient in classifying a broad range of persistent organic pollutants (POPs).¹⁷ The proper identification of the contaminants using such a system would provide knowledge that would then inform the rational development of a decontamination strategy.

Our group has previously employed both β -cyclodextrin and γ -cyclodextrin in the development of array-based detection systems for the sensing and classification of a wide variety of environmental toxicants and POPs.¹⁸ The sensing strategy is based on cyclodextrin promoted analyte-to-fluorophore energy transfer as well as on analyte-induced fluorescence modulation. This fluorescence modulation relies on doping of the free fluorophore into the cyclodextrin solution prior to analyte addition, which often leads to binding of the fluorophore in the cyclodextrin and reduces the cyclodextrin's ability to bind the target analyte. As such, introduction of the analyte to the fluorophore-cyclodextrin solution requires the analyte-cyclodextrin association constants to be higher than the fluorophore-cyclodextrin constants in order to achieve binding (Figure 1A), or it requires the formation of higher order association complexes between the analyte, cyclodextrin and fluorophore (*i.e.* ternary complex formation) (Figure 1B). Such higher order association complexation is probable only for γ -cyclodextrin.¹⁹ β -cyclodextrin, by contrast, has been extensively reported to participate

in the formation of binary association complexes,²⁰ and ternary inclusion complexes with β -cyclodextrin are less reported.²¹

Figure 1. Schematic illustration of the advances in this work compared to previously published work.

Although in our previous work we have successfully distinguished between classes of analytes, the results tend to cluster structurally similar analytes and analyte derivatives near each other. Herein, we report the development of a highly selective array-based detection system using fluorophore-functionalized perbenzylated β -cyclodextrin sensors as the key components, which directly enables binary complex formation between the fluorophore-cyclodextrin and target analyte (Figure 1C). Each individual sensor is highly selective towards a specific isomer/analogue within a group of structurally similar analytes, which enables the array to distinguish isomers and structural analogues with high efficiency. Three classes of isomeric analytes and two classes of structurally similar analytes have been successfully classified based on this strategy, with a classification accuracy of 100% in every case. High sensitivity is demonstrated as well, with limits of detection approaching or surpassing literature-

reported levels of concern. Finally, preliminary efforts at using this system for accurate identification of binary analyte mixtures are also reported.

RESULTS AND DISCUSSION

We employed a series of three cyclodextrin-based supramolecular sensors (Figure 2) for the detection of a broad variety of small molecule analytes (Figure 3). In these sensors, the perbenzylated β -cyclodextrin cavity acts as the receptor domain, and the attached fluorophore units act as the transducers, which are responsible for fluorescence-based responses to changes in their environment in the presence of the target analyte. The covalent attachment strategy used in sensors **S2** and **S3**, with one and two degrees of functionalization on the primary rim, respectively, ensures the close proximity of the fluorophore units to the cyclodextrin receptor cavity, thereby facilitating productive fluorophore-analyte interactions. In contrast, sensor **S1** is a non-covalent combination of the perbenzylated β -cyclodextrin and fluorophore **4**, and is included to enable a direct determination of the benefits of covalent attachment in such sensor design.

Figure 2. Structures of sensors **S1** – **S3**.

Figure 3. Structures of small molecule analytes **5** – **26**.

The synthesis of supramolecular hosts **S2** and **S3** is shown in Scheme 1. Perbenzylated β -cyclodextrin is obtained from reacting β -cyclodextrin with excess benzyl chloride at room temperature in DMSO in the presence of excess sodium hydride.²² Regioselective debenzylation of the primary rim is effected by treating the perbenzylated β -cyclodextrin with DIBAL-H in toluene (ways to control the selectivity to achieve mono vs. di-debenzylation are discussed in the ESI).²³ This is followed by Steglich esterification²⁴ with the acid derivative of fluorophore **4**, yielding mono- and di-functionalized sensors **S2** and **S3**. New supramolecular compounds **S2** and **S3** were fully characterized by ^1H -NMR, ^{13}C -NMR, MALDI-TOF mass spectrometry, and UV-visible and fluorescence spectroscopy.

Scheme 1. Synthesis of supramolecular hosts **S2** and **S3**.

The fluorescence emission responses of sensors **S1**, **S2** and **S3** were investigated in varying solvent systems, with the goal of ensuring full dissolution of the sensor (requiring some DMSO) while enabling strong binding of analytes in the cyclodextrin host (optimal for aqueous environments). While sensor **S1** showed a high fluorescence emission in predominantly aqueous solutions, sensors **S2** and **S3** displayed very little changes with changes in solvent composition. This led us to choose an 80:20 water-DMSO mixture as our sensing solvent. We note that the covalent strategy of the fluorophore used in sensors **S2** and **S3** led to a dramatic reduction of the fluorescence emission compared to the free fluorophore in **S1** (Figure 4). This fluorescence decrease is in agreement with literature precedent in analogous systems, and is offset by the markedly improved fluorescence modulation results in the presence of various analytes (*vide infra*).²⁵

Figure 4. Fluorescence emission spectra of supramolecular hosts **S1** – **S3** (1 μ M) (inset shows the fluorescence of **S2** and **S3** in more detail) in 80:20 water-DMSO solution. (λ_{ex} = 320 nm; 3 nm excitation slit width; 3 nm emission slit width).

The choice of perbenzylated β -cyclodextrin as a receptor over that of β -cyclodextrin is due to the stronger binding of organic guest molecules as a result of its extended hydrophobic cavity.²⁵ In particular, a comparison of association constant values of analyte **5** revealed a 1000-fold increase in the binding constant with perbenzylated β -cyclodextrin over that of the naturally occurring β -cyclodextrin, and this binding constant is even higher in the fluorophore-functionalized cyclodextrins **S2** and **S3** (Table 1). These binding constants are orders of magnitude higher than highest literature-reported binding constants for analyte **5** in β -cyclodextrin (K_a = 50-215 M^{-1}).²⁶ In general, higher association constants for the binding of an analyte in a sensor are known to lead to improved sensor performance.²⁷

Table 1. Association constants of analyte **5** in β -cyclodextrin^a and in perbenzylated β -cyclodextrin^b, **S2**^b & **S3**^b.

Host	Association Constant (M ⁻¹)
β -cyclodextrin	0.2×10^2
Perbenzylated β -cyclodextrin	$3.6 (0.1) \times 10^4$
S2	$4.8 (0.5) \times 10^4$
S3	$24.9 (0.5) \times 10^4$

^a Association constant reported in the literature in aqueous solution.

^b Association constants calculated using ¹H NMR titrations in 80:20 water-DMSO mixture.

Similarly, in this case, strong binding of analytes **5-8** in hosts **S1- S3** induced marked changes in the resulting fluorescence emission due to proximity-induced interactions between the analyte and the fluorophore. These fluorescence modulation changes were quantified according to Equation 1, below:

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

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

Although the fluorescence response was essentially unchanged with analyte addition in the case of **S1** (leading to modulation values near 1.00 in every case), significant differences in the response patterns of sensors **S2** and **S3** with analyte addition were observed (Table 2). An example of analyte-induced fluorescence modulation for analyte **8** is shown in Figure 5 and highlights the small but distinct

fluorescence changes observed for **S2** and **S3**. The fluorescence signals of sensors **S1-S3** in the presence of analytes **5-8** were subjected to linear discriminant analysis, and enabled 100% selectivity between the different aromatic alcohol isomers (Figure 6). This selectivity is particularly noteworthy as such isomers are challenging to separate using other chemical techniques.²⁸

Table 2. Fluorescence modulation of supramolecular sensors in the presence of aromatic alcohol analytes **5 – 8**.

Analyte	S1	S2	S3
5	1.00 ± 0.00	1.04 ± 0.01	0.98 ± 0.01
6	1.01 ± 0.00	0.82 ± 0.01	0.88 ± 0.01
7	0.99 ± 0.00	0.90 ± 0.00	1.05 ± 0.02
8	1.01 ± 0.01	0.87 ± 0.01	0.75 ± 0.01

a Results were calculated using Equation 1. All results represent an average of at least 3 trials.

Figure 5. Fluorescence emission of (A) sensor **S1**; (B) sensor **S2**; and (C) sensor **S3** in the presence of analyte **8**. (λ_{ex} = 320 nm; 3 nm excitation slit width; 3 nm emission slit width).

Figure 6. Linear discriminant analysis showing 100% differentiation between analytes **5 – 8** based on their interactions with supramolecular hosts **S1 – S3**.

The binding of other structural isomers and analogues in supramolecular hosts **S1-S3** also led to noticeable, analyte-specific changes in the fluorescence emission (Table 3), with some key results highlighted in Figures 7-10.

Table 3. Fluorescence modulation of sensors **S1 – S3** in the presence of analytes **9 – 26**.

Analyte	S1	S2	S3
9	1.01 ± 0.00	0.89 ± 0.00	1.07 ± 0.05
10	1.01 ± 0.00	0.90 ± 0.00	0.97 ± 0.01
11	1.01 ± 0.00	0.99 ± 0.03	0.77 ± 0.06
12	0.99 ± 0.00	0.89 ± 0.00	1.14 ± 0.01
13	1.00 ± 0.00	0.93 ± 0.01	1.33 ± 0.03
14	1.01 ± 0.00	0.95 ± 0.01	1.07 ± 0.04
15	0.98 ± 0.01	1.17 ± 0.01	1.35 ± 0.05
16	0.99 ± 0.01	1.08 ± 0.01	1.04 ± 0.05
17	1.00 ± 0.00	1.01 ± 0.01	0.94 ± 0.02
18	1.05 ± 0.00	1.06 ± 0.00	0.93 ± 0.02
19	0.98 ± 0.00	1.09 ± 0.01	0.95 ± 0.02
20	1.00 ± 0.00	0.99 ± 0.01	1.01 ± 0.01
21	1.03 ± 0.01	1.03 ± 0.02	0.89 ± 0.01

22	1.03 ± 0.00	1.06 ± 0.06	0.85 ± 0.01
23	1.01 ± 0.01	1.02 ± 0.04	0.98 ± 0.03
24	1.01 ± 0.00	1.07 ± 0.04	0.89 ± 0.02
25	1.05 ± 0.00	0.56 ± 0.01	0.98 ± 0.01
26	1.00 ± 0.01	0.92 ± 0.03	1.14 ± 0.02

a Fluorescence modulation results were calculated using Equation 1. All results represent an average of at least 3 trials.

Figure 7. (A) Fluorescence response of host **S1** in the presence of analytes **9 – 12**; (B) Linear discriminant analysis of the fluorescence responses, leading to 100% differentiation of the analyte signals. ($\lambda_{\text{ex}} = 320 \text{ nm}$; 3 nm excitation slit width; 3 nm emission slit width).

Figure 8. (A) Fluorescence response of host **S2** in the presence of analytes **13 – 16**;
(B) Linear discriminant analysis of the fluorescence responses, leading to 100%
differentiation of the analyte signals. ($\lambda_{\text{ex}} = 320$ nm; 3 nm excitation slit width; 3 nm
emission slit width).

Figure 9. (A) Fluorescence response of host **S3** in the presence of analytes **17 – 21**;
(B) Linear discriminant analysis of the fluorescence responses, leading to 100%
differentiation of the analyte signals. ($\lambda_{\text{ex}} = 320$ nm; 3 nm excitation slit width; 3 nm
emission slit width).

Figure 10. (A) Fluorescence response of host **S2** in the presence of analytes **22 – 26**; (B) Linear discriminant analysis of the fluorescence responses, leading to 100% differentiation of the analyte signals. ($\lambda_{\text{ex}} = 320$ nm; 3 nm excitation slit width; 3 nm emission slit width).

The sensor **S1** shows a fluorescence modulation value close to 1.00 for all the tested analytes (see Figure 5A for an example), indicating minimal to no effect on the fluorescence emission of the fluorophore with the introduction of the analyte. In contrast to this, fluorescence modulation values measured for sensors **S2** and **S3** are significantly different from that of **S1**, and display widespread variability between different classes of analytes as well as within each analyte class. These results clearly demonstrate the effect of the sensor architecture, and in particular the effects of covalent fluorophore attachment and the number of fluorophore transducer units. The covalent attachment ensures close proximity between the cyclodextrin-bound analyte and the fluorophore moiety(ies), causing substantial modulation of the fluorescence emission signal.

Analytes **9-12** represent a class of aliphatic alcohols consisting of cyclohexylmethanol (**11**) and its isomers. These compounds are widely used as alkene precursors,²⁹ and a structurally similar analogue was part of a recent chemical spill,³⁰ making them important targets for detection. While all the analytes are structural isomers, analytes **10** and **12** are also stereoisomers. Distinct fluorescence modulation values are noted for sensor **S3** in combination with stereoisomers **10** and **12**, highlighting the power of the cyclodextrin-based supramolecular sensor in differentiating even small structural changes. Overall, the uses of sensors **S1-S3** in

combination with these analytes enabled 100% differentiation using linear discriminant analysis (Figure 6).

Analytes **13-16** represents aromatic pesticide *p,p*-DDT (compound **15**), its known metabolites DDE (compound **13**) and DDD (compound **14**),³¹ and its often co-occurring structural isomer *o,p*-DDT (compound **16**).³² These compounds are suspected carcinogens³³ and toxicants,³⁴ and are therefore important targets for detection. Although the analogues selected are extremely structurally similar, we nonetheless achieved 100% accurate classification for these analytes using supramolecular sensors **S1-S3** and linear discriminant analysis (Figure 7). Interestingly, although sensor **S3** demonstrated nearly identical fluorescence modulation values in response to analytes **13** and **15**, sensor **S2** was able to clearly differentiate between those two analytes. These results illustrate the fact that altering the degree of functionalization of the supramolecular sensor alters its response for a target analyte.

Analytes **17-21** represent aliphatic *n*-hexane (compound **17**), its commonly occurring structural isomers (compounds **18-20**, generated in 10-30% yield from industrial production of hexane)³⁵ and its cyclopentane analogue (compound **21**). The fact that hexanes co-occur as isomeric mixtures of compounds **17-20** complicates their accurate characterization as well as fuel applications that rely on such characterization.³⁶ Using this supramolecular sensing strategy, 100% accurate classification between these analytes has been achieved.

Analytes **22-26** represent a class of (POPs) called polychlorinated biphenyls (PCBs), which cause neurotoxicity³⁷ and endocrine disruption.³⁸ As a result, the use of

PCBs has been banned in many countries; however, their extreme environmental persistence means that significant amounts of PCBs are still found in the environment today.³⁹ 100% accurate classification has been achieved for these analytes via this strategy, which is particularly relevant because these analytes have widely disparate toxicities.

The ability of this detection method to generate well-separated signals was further investigated by generating an array with all analytes from all classes. In this case, the array exhibited well-separated clusters based on compound class, as well as excellent separation within each class. Overall, 100% accurate identification was obtained (see ESI for more details).

The limits of detection for each sensors **S1**, **S2** and **S3** for each class of analytes were calculated to determine their ability to sense analyte concentrations at or near environmental levels of concern and literature-reported levels of toxicity. In every case, the calculated limits of detection were at or lower than the literature reported limits of concern for these analytes (Table 4). These results illustrate the high sensitivity of this detection method.

Practical applications of this system require the capability to identify analyte **mixtures**, because environmental contamination scenarios almost never involve a single chemical contaminant. To that end, preliminary work focused on identification of 1:1 binary mixtures of aromatic alcohol analytes **5-8**. Using the same supramolecular sensors and the same linear discriminant analytical techniques, 83% accurate identification of the 1:1 binary mixtures was obtained (Figure 11). Interestingly, the mixture of analytes **5 + 7** is grouped near the mixtures of analytes **6**

+ **8** and **5 + 8**, which reduces the overall classification accuracy. This kind of co-clustering of analyte groups has been observed in literature precedent reports of array-based sensing⁴⁰, and can possibly be attributed to similar sensor responses originating from competing interactions between each component of the mixture. Other than those combinations, the other mixtures demonstrated excellent signal separation and accurate identification. Current work in our group is focused on improving classification accuracy of analyte mixtures, expanding such techniques to multiple analyte classes, and moving from binary mixtures to ternary and even quaternary mixtures of analytes.

Table 4. Calculated limits of detection and comparisons to known levels of concern

Analytes	Sensors	LOD calculated (μM)	Limit of concern (μM)
5	S2	0.39	a
6	S1	0.51	21.27 ⁴¹
6	S3	2.20	21.27 ⁴¹
9	S3	4.97	437.87 ⁴²
11	S1	8.34	a
11	S2	11.79	a
15	S1	1.17	2.82 ⁴³
15	S2	1.85	2.82 ⁴³
15	S3	26.30	2.82 ⁴³
18	S1	2.20	5801.81 ⁴⁴
19	S2	15.74	5801.81 ⁴⁵
21	S3	19.82	a
25	S1	0.29	1.71 ⁴⁶
26	S2	0.88	1.00 ⁴⁴
26	S3	4.59	1.00 ⁴⁴

^a Limits of concern have not been established for these compounds.

Figure 11. Linear discriminant analysis results of binary mixtures of analytes **5 – 8**.

CONCLUSION

In conclusion, we have developed an efficient array-based detection strategy for isomeric and analogous analytes. The array employs three architecturally unique perbenzylated β -cyclodextrin-fluorophore sensors for identification of a particular isomer within a class of isomeric or structurally similar analytes. The binding of analytes to the cyclodextrin induces a distinct change in the fluorescence emission of the attached fluorophore units, which is then statistically translated into array clusters of maximum separation via linear discriminant analysis (LDA). We demonstrate a 100% successful classification of three isomeric (aromatic alcohols, aliphatic alcohols, aliphatic hexanes) and two analogous (DDT pesticides, PCB congeners) classes of analytes. Sensitivity measurements highlight that limits of detection are at or near literature-reported levels of concern. Finally, preliminary work on binary mixtures demonstrated promising levels of selectivity, with 83% accuracy obtained. Current work in our laboratory is focused on expanding the classes of analytes detectable via this system, improving analyte mixture identification, and developing a practical

cyclodextrin-based detection device. The results of these and other investigations will be reported in due course.

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Supporting Information

Array-based detection of isomeric and analogous analytes employing synthetically modified fluorophore attached β -cyclodextrin derivatives

METHODS AND MATERIALS

All of the reagents were obtained from Sigma Aldrich or Fisher Scientific and used without further purification, unless otherwise noted. β -cyclodextrin was dried in the oven prior to use. Reagent grade solvents (99.9% purity) were used for the synthetic reactions. Column chromatography was performed in a Yamazen AKROS-Automatic TLC Smart Flash Chromatography System. ^1H and ^{13}C NMR spectra were recorded in a 400 MHz Bruker AVANCE and 500 MHz Varian NMR spectrometer, with assistance from Dr. Al Bach. Mass spectra were recorded in a Bruker Omnix MALDI-TOF instrument with 2,5-dihydroxybenzoic acid as a matrix at the Department of Chemistry Instrumentation Facility (DCIF) at the Massachusetts Institute of Technology (MIT), with samples run by Dr. Li Li. All of the fluorescence measurements were performed using a Shimadzu RF 5301 spectrofluorophotometer. Both the excitation and emission slit widths were 3 nm. All of the fluorescence spectra were integrated vs. wavenumber on the X-axis using Origin Pro Version 9.1 software. All arrays were generated using SYSTAT Version 13.

DETAILED PROCEDURES

DETAILED SYNTHETIC PROCEDURES

Overall Synthetic Scheme:

Reaction 1: Synthesis of Perbenzylated β-Cyclodextrin:

To a stirred solution of oven-dried β-cyclodextrin (2.00 g, 1.76 mmol, 1.0 eq.) in DMSO (100 mL) under nitrogen, sodium hydride (2.60 g, 65 mmol, 36 eq.) was added carefully. The solution was allowed to stir for one hour at room temperature, after which time benzyl chloride (18.5 mL, 65 mmol, 36 eq.) was added over the course of one hour. The reaction mixture was stirred for 18 hours at room temperature,

followed by the addition of methanol (20 mL). The reaction mixture was then diluted with water (200 mL) and extracted with diethyl ether (3 x 200 mL). The combined organic layers were washed with brine (200 mL), dried with anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was purified via column chromatography (25-40% v/v gradient elution of ethyl acetate/hexanes) to obtain a white foamy compound **1** (3.6 g, 70 % yield) after being dried under high vacuum. ¹H NMR (400 MHz, CDCl₃): δ = 3.52 (dd, ³J_{2,3} = 9.2 Hz, ³J_{2,1} = 3.3 Hz, 7 H; 2-H), 3.58 (d, ²J = 10.6 Hz, 7 H; 6-H), 3.98-4.10 (m, 28 H; 3-H, 4-H, 5-H, 6-H), 4.39, 4.43 (AB, J_{A,B} = 12.2 Hz, 14 H; CH₂Ph), 4.50, 4.54 (AB, J_{A,B} = 12.8 Hz, 14 H; CH₂Ph), 4.81, 5.11 (AB, J_{A,B} = 11.0 Hz, 14 H; CH₂Ph), 5.22 (d, ³J_{1,2} = 3.3 Hz, 7 H; 1-H), 7.15-7.30 (m, 105 H; aromatic-H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 69.2, 71.4, 72.6, 73.2, 75.4, 78.6, 78.7, 80.8, 98.4, 126.9-128.3, 138.1, 138.3, 139.2 ppm; MS (MALDI-TOF): m/z = 3050.49 [M+Na]⁺ (Calculated for C₁₈₉H₁₉₆O₃₅ + Na⁺ = 3050.55).

Reaction 2: Synthesis of Mono-debenzylated β-cyclodextrin:

To a stirred solution of compound **1** (600 mg, 0.2 mmol, 1.0 eq.) in anhydrous toluene (65 mL) under nitrogen, diisobutylaluminum hydride (DIBAL-H) (4.7 mL, 7.0 mmol, 35 eq.) was added dropwise to a final concentration of 0.1 M. The reaction

mixture was allowed to stir for 2 hours at room temperature, after which the complete disappearance of starting material was observed via TLC analysis (25% v/v ethyl acetate/hexane). The reaction mixture was cooled to 0 °C and hydrolyzed via the addition of 10% aqueous HCl (15 mL) for 15 minutes. The crude product was extracted with ethyl acetate (100 mL), treated with anhydrous Na₂SO₄ and dried under reduced pressure. Purification via column chromatography (1:3 ethyl acetate/hexane gradient elution) led to a white compound **4** (250 mg, 40 % yield). ¹H NMR (400 MHz, CDCl₃): δ = 2.48 (br s, 1 H; OH), 3.34-4.07 (m, 42 H; 7x2-H, 7x3-H, 7x4-H, 7x5-H, 14x6-H), 4.27-4.51 (m, 24H; CH₂Ph), 4.60-4.75 (m, 10H; CH₂Ph), 4.88-5.01 (m, 6H; 6x1-H), 5.08-5.18 (m, 4 H; CH₂Ph), 5.25 (dd, ³J_{1,2} = 12.0, 4.0 Hz, 2 H; CH₂Ph), 5.36 (d, ³J_{1,2} = 4.0 Hz, 1 H; 1x1-H), 7.04-7.30 (m, 100 H; aromatic-H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 61.6, 68.8, 69.2, 69.3, 69.4, 71.4, 71.5, 71.6, 71.7, 71.7, 71.8, 71.9, 72.5, 72.6, 72.7, 72.7, 72.9, 73.0, 73.3, 73.4, 73.4, 74.8, 75.0, 75.1, 75.3, 75.8, 75.9, 75.9, 76.0, 77.4, 77.7, 78.1, 78.8, 79.0, 79.1, 79.5, 79.6, 79.9, 80.1, 80.9, 81.0, 81.0, 81.1, 98.0, 98.3, 98.4, 98.4, 98.6, 98.8, 98.9, 127.0-128.4, 137.9, 138.1, 138.2, 138.2, 138.2, 138.3, 138.3, 138.4, 138.5, 138.5, 139.0, 139.1, 139.3, 139.3, 139.4, 139.4 ppm; MS (MALDI-TOF): m/z = 2960.29 [M+Na]⁺ (Calculated for C₁₈₂H₁₉₀O₃₅ + Na = 2960.43).

Reaction 3: Synthesis of Di-debenzylated β-cyclodextrin:

To a stirred solution of compound **1** (1.2 g, 0.4 mmol, 1.0 eq.) under nitrogen, DIBAL-H (4.0 mL, 6.0 mmol, 15 eq.) was added dropwise. The reaction mixture was stirred for 6 hours at 50 °C until a complete disappearance of starting material was observed via TLC analysis. After an additional 15 minutes of stirring, the reaction mixture was cooled to 0 °C and hydrolyzed by vigorously stirring with 10 % aqueous HCl (15 mL) for 20 minutes. The crude product was extracted with ethyl acetate (100 mL), treated with anhydrous Na₂SO₄ and dried under reduced pressure. Purification via column chromatography (1:3 ethyl acetate/hexanes) led to a white compound **5** (566 mg, 50 % yield). ¹H NMR (400 MHz, CDCl₃): δ = 2.69 (br s, 1 H ; OH), 2.78 (br s, 1 H; OH), 3.44-3.54 (m, 5 H; 5x2-H), 3.60-4.15 (m, 37 H; 2x2-H, 7x3-H, 7x4-H, 7x5-H, 14x6-H), 4.44-4.88 (m, 33 H; CH₂Ph), 4.89 (d, ³J_{1,2} = 3.3 Hz, 1 H ; 1-H), 4.98 (d, ³J_{1,2} = 3.7 Hz, 1H ; 1-H), 5.00 (d, ³J_{1,2} = 4.0 Hz, 1 H; 1-H), 5.02 (d, ³J_{1,2} = 3.4 Hz, 1 H ; 1-H), 5.04 (d, ³J_{1,2} = 3.5 Hz, 1 H ; 1-H), 5.06 (d, ²J = 12.3 Hz, 1 H ; CH₂Ph), 5.21-5.25 (m, 3 H; 3xCH₂Ph), 5.30 (d, ²J = 10.7 Hz, 1 H;CH₂Ph), 5.56 (d, ³J_{1,2} = 3.8 Hz, 1 H; 1-H), 5.67 (d, ³J_{1,2} = 3.7 Hz, 1 H; 1-H), 7.12-7.33 (m, 95H; aromatic-H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 61.6, 69.5, 69.6, 71.2, 71.6, 72.0, 72.1, 72.9, 73.2,

73.25, 73.3, 73.9, 74.1, 76.1, 76.4, 77.6, 79.0, 79.7, 80.6, 80.9, 81.0, 81.6, 81.7, 97.6, 97.7, 98.2, 126.3-128.3, 137.7, 137.8, 137.9, 138.2, 138.6, 137.7, 139.2 ppm; MS (MALDI-TOF): $m/z = 2870.1$ $[M+Na]^+$ (Calculated for $C_{175}H_{184}O_{35} + Na = 2870.31$).

Reaction 4: Synthesis of Sensor **S2**:

A mixture of compound **4** (100 mg, 0.034 mmol, 1.0 eq.), compound **6** (10.5 mg, 0.04 mmol, 1.17 eq.), *N,N'*-dicyclohexylcarbodiimide (DCC) (8.3 mg, 0.04 mmol, 1.17 eq.) and 4-dimethylaminopyridine (DMAP) (0.5 mg, 0.004 mmol, 0.1 eq.) in dichloromethane (1 mL) was stirred at 50 °C for 24 hrs. The mixture was filtered, treated with 5% aqueous acetic acid (2 x 3 mL) and extracted with dichloromethane (2 x 4 mL). The combined organic layer was dried under anhydrous Na_2SO_4 and subjected to solvent removal under reduced pressure. The crude product was purified via column chromatography (1:3 ethyl acetate/hexanes) to yield a white amorphous compound **2** (32 mg, 30% yield). 1H NMR (500 MHz, d_6 -acetone): $\delta = 2.31$ (s, 3 H; ArCH₃), 2.62 (m, 2 H; CH₂FL3), 2.93 (t, $^3J_{1,2} = ^3J_{1,2'} = 10.0$ Hz, 2 H; CH₂CHFL3), 3.43-3.50 (m, 7 H; 2-H), 3.62-3.74 (m, 7 H; 6-H), 3.84 (br t, 2 H; 6-H), 3.89 (s, 3 H; OCH₃), 3.92-4.16 (m, 26 H; 3-H, 4-H, 5-H, 6-H), 4.40-4.62 (m, 26 H; CH₂Ph), 4.75-4.78 (m, 7 H; CH₂Ph), 5.09-5.13 (m, 7 H; CH₂Ph), 5.16 (d, $^3J_{1,2} = 3.5$ Hz, 1 H; 1-H), 5.27 (dd, $^3J_{1,2} = 10, 3.5$ Hz, 2 H; 1-H), 5.30 (m, 3 H; 1-H), 5.33 (d, $^3J_{1,2} = 3.5$ Hz, 1 H;

1-H), 6.02 (s, 1 H; $CH=CCH_3$), 6.86 (s, 1 H; ArH), 7.12-7.33 (m, 80 H; PhH), 7.48 (s, 1 H; ArH) ppm; ^{13}C NMR (125 MHz, d_6 -acetone): δ = 17.8, 25.3, 33.6, 55.7, 63.5, 69.5, 69.8, 71.7, 71.9, 72.4, 72.7, 73.0, 75.2, 78.3-79.4, 80.8-81.1, 97.8-98.0, 98.2, 98.7, 98.7, 111.5, 112.8, 124.5, 125.6, 126.8, 127.29-128.25, 138.6, 138.7-138.8, 139.5-139.6, 152.8, 154.3, 160.1, 160.6, 172.0 ppm; MS (MALDI-TOF): m/z = 3204.57 $[M+Na]^+$ (Calculated for $C_{196}H_{202}O_{39} + Na = 3204.67$).

Reaction 5: Synthesis of Sensor **S3**:

A mixture of compound **5** (100 mg, 0.035 mmol, 1.0 eq.), compound **6** (21.0 mg, 0.08 mmol, 2.34 eq.), *N,N'*-dicyclohexylcarbodiimide (16.5 mg, 0.08 mmol, 2.34 eq.) and 4-dimethylaminopyridine (1.1 mg, 0.008 mmol, 0.2 eq.) in dichloromethane (1 mL) was stirred at 50 °C for 24 hrs. The mixture was filtered, treated with 5% aqueous acetic acid (2 x 3 mL) and extracted with dichloromethane (2 x 4 mL). The combined organic layer was dried under anhydrous Na_2SO_4 and subjected to solvent removal under reduced pressure. The crude product was purified via column chromatography (1:3 ethyl acetate: hexanes) to lead to a white amorphous compound **3** (30 mg, 25 % yield). 1H NMR (500 MHz, d_6 -acetone): δ = 2.31 (s, 6 H; $ArCH_3$), 2.62 (m, 4 H; $CHFL_3$), 2.93 (m, 4 H; $CHCHFL_3$), 3.44-3.51 (m, 7 H; 2-H), 3.62-3.74 (m, 7 H; 6-H), 3.82-3.89 (multiplet overlapped, 4 H; 6-H), 3.89 (singlet overlapped, 6 H; OCH_3),

3.94-4.16 (m, 24 H; 3-H, 4-H, 5-H, 6-H), 4.41-4.64 (m, 26H; CH_2Ph), 4.74-4.78 (m, 6H; CH_2Ph), 5.08-5.12 (m, 6H; CH_2Ph), 5.22 (dd, $^3J_{1,2} = 8.5, 3.5$ Hz, 2H; 1-H), 5.26 (m, 3 H; 1-H), 5.29 (m, 2 H; 1-H), 6.01 (s, 2 H; $CH=CCH_3$), 6.86 (s, 2 H; ArH), 7.06-7.30 (m, 80 H; PhH), 7.46 (d, $^3J_{1,2} = 6.5$ Hz, 2H; ArH) ppm; ^{13}C NMR (125 MHz, d_6 -acetone): $\delta = 17.8, 24.6, 25.2-25.4, 25.6, 25.8, 30.6, 32.1, 33.5, 34.1, 55.7, 63.5, 69.3-69.8, 71.6-73.1, 75.2, 78.3-79.4, 80.7-81.0, 97.9-98.7, 111.5, 112.7, 125.4-125.5, 126.8, 127.3-128.3, 138.6, 138.7-138.8, 139.4-139.6, 152.7, 154.3, 160.0, 160.6, 172.1$ ppm; MS (MALDI-TOF): $m/z = 3358.82 [M+Na]^+$ (Calculated for $C_{203}H_{208}O_{43} + Na = 3358.40$).

DETAILED PROCEDURES FOR FLUORESCENCE MODULATION EXPERIMENTS

Fluorescence emission spectra were obtained using a Shimadzu RF-5301PC spectrophotofluorimeter with 3 nm excitation and 3 nm emission slit widths. In a quartz cuvette, 0.5 mL of **S1**, **S2**, or **S3** solutions (5 μ M in DMSO) and 2 mL of deionized water were combined. Then, the solution was excited at 320 nm, and the fluorescence emission spectra were recorded. Repeat measurements were recorded for four separate trials.

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 10 μL of analyte (1 mg/mL in THF), and Fl_{blank} is the integrated fluorescence emission of the fluorophore in the absence of the analyte.

DETAILED PROCEDURES 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: **S1**, **S2**, and **S3**
- (d) Long-range statistics: Mahal

DETAILED PROCEDURES 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 cyclodextrin-analyte combination. In a quartz cuvette, 0.5 mL of **S1**, **S2**, or **S3** solutions (5 μM in DMSO) and 2 mL of deionized (DI) water were combined. Then, the solution was excited at 320 nm, and the fluorescence emission spectra were recorded starting at 330 nm. Six repeat measurements were taken.

Next, 2 μL 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 4 μL of analyte, 6 μL of analyte, 8 μL of analyte, 10 μL of analyte, 12 μL of analyte, 14 μL of analyte, 16 μL of analyte, 18 μL of analyte, 20 μL 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 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.

The limit of detection is defined according to Equation 2:

$$\text{LOD} = 3(\text{SD}_{\text{blank}})/m \quad (\text{Eq. S2})$$

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 calculated in μM .

SUMMARY TABLES

FLUORESCENCE MODULATION SUMMARY TABLES

Analyte	S1	S2	S3
benzyl alcohol	1.00 ± 0.00	1.04 ± 0.01	0.98 ± 0.01
<i>o</i> -cresol	1.01 ± 0.00	0.82 ± 0.01	0.88 ± 0.01
<i>m</i> -cresol	0.99 ± 0.00	0.90 ± 0.00	1.05 ± 0.02
<i>p</i> -cresol	1.01 ± 0.01	0.87 ± 0.01	0.75 ± 0.01

Analyte	S1	S2	S3
1-methylcyclohexanol	1.01 ± 0.00	0.89 ± 0.00	1.07 ± 0.05
<i>cis</i> -2-methylcyclohexanol	1.01 ± 0.00	0.90 ± 0.00	0.97 ± 0.01
cyclohexylmethanol	1.01 ± 0.00	0.99 ± 0.03	0.77 ± 0.06
<i>trans</i> -2-methylcyclohexanol	0.99 ± 0.00	0.89 ± 0.00	1.14 ± 0.01

Analyte	S1	S2	S3
DDD	1.00 ± 0.00	0.93 ± 0.01	1.33 ± 0.03
DDE	1.01 ± 0.00	0.95 ± 0.01	1.07 ± 0.04
<i>o,p</i> -DDT	0.99 ± 0.01	1.08 ± 0.01	1.04 ± 0.05
<i>p,p</i> -DDT	0.98 ± 0.01	1.17 ± 0.01	1.35 ± 0.05

Analyte	S1	S2	S3
<i>n</i> -hexanes	1.00 ± 0.00	1.01 ± 0.01	0.94 ± 0.02
2-methylpentane	1.05 ± 0.00	1.06 ± 0.00	0.93 ± 0.02
3-methylentane	0.98 ± 0.00	1.09 ± 0.01	0.95 ± 0.02
2,3-dimethylbutane	1.00 ± 0.00	0.99 ± 0.01	1.01 ± 0.01
1-methylcyclopentane	1.03 ± 0.01	1.03 ± 0.02	0.89 ± 0.01

Analyte	S1	S2	S3
PCB3	1.03 ± 0.00	1.06 ± 0.06	0.85 ± 0.01
PCB29	1.01 ± 0.01	1.02 ± 0.04	0.98 ± 0.03
PCB52	1.01 ± 0.00	1.07 ± 0.04	0.89 ± 0.02
PCB77	1.05 ± 0.00	0.56 ± 0.01	0.98 ± 0.01
PCB209	1.00 ± 0.01	0.92 ± 0.03	1.14 ± 0.02

LIMIT OF DETECTION SUMMARY TABLE

Analyte	Host	Equation	R ²	LOD (μM)
<i>p,p</i> -DDT	S1	$y = 0.0094x + 1.0385$	0.939	0.39
<i>p,p</i> -DDT	S2	$y = 0.011x + 0.971$	0.9406	0.51
<i>p,p</i> -DDT	S3	$y = 0.0188x + 0.9592$	0.9547	2.20
<i>o</i> -Cresol	S1	$y = 0.0018x + 1.0195$	0.9748	4.97
Benzyl alcohol	S2	$y = 0.0032x + 0.932$	0.8521	8.34
<i>o</i> -Cresol	S3	$y = -0.0026x + 0.7242$	0.9893	11.79
Cyclohexylmethanol	S1	$y = 0.01x + 0.9866$	0.9708	1.17
Cyclohexylmethanol	S2	$y = -0.0031x + 0.9648$	0.9405	1.85
1-Methylcyclohexanol	S3	$y = 0.0012x + 0.942$	0.9236	26.30
2-Methylpentane	S1	$y = 0.0026x + 0.9776$	0.9555	2.20
3-Methylpentane	S2	$y = 0.0017x + 1.0775$	0.9864	15.74
1-Methylcyclopentane	S3	$y = 0.0038x + 0.7209$	0.9421	19.82
PCB 77	S1	$y = 0.0116x + 1.0153$	0.8832	0.29
PCB 209	S2	$y = -0.0077x + 0.8402$	0.9655	0.88
PCB 209	S3	$y = 0.0079x + 1.0621$	0.8686	4.59

SUMMARY TABLES FOR ARRAYS

All analytes

Jackknifed Classification Matrix

	1-methylcyclohexanol	1-methylcyclopentane	2,3-dimethylbutane	2-methylpentane	3-methylpentane	DDD	DDE
1-methylcyclohexanol	4	0	0	0	0	0	0
1-methylcyclopentane	0	4	0	0	0	0	0
2,3-dimethylbutane	0	0	4	0	0	0	0
2-methylpentane	0	0	0	4	0	0	0
3-methylpentane	0	0	0	0	4	0	0
DDD	0	0	0	0	0	4	0
DDE	0	0	0	0	0	0	4
benzyl alcohol	0	0	0	0	0	0	0
cis-2methylcyclohexanol	0	0	0	0	0	0	0
cyclohexylmethanol	0	0	0	0	0	0	0
m-cresol	0	0	0	0	0	0	0
n-hexanes	0	0	0	0	0	0	0
o-cresol	0	0	0	0	0	0	0
opDDT	0	0	0	0	0	0	0
p-cresol	0	0	0	0	0	0	0
pcb209	0	0	0	0	0	0	0
pcb29	0	0	0	0	0	0	0
pcb3	0	0	0	0	0	0	0
pcb52	0	0	0	0	0	0	0
pcb77	0	0	0	0	0	0	0
ppDDT	0	0	0	0	0	0	0
trans-2methylcyclohexanol	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	benzyl alcohol	cis-2methylcyclohexanol	cyclohexylmethanol	m-cresol	n-hexanes	o-cresol	opDDT	p-cresol	pcb209
1-methylcyclohexanol	0	0	0	0	0	0	0	0	0
1-methylcyclopentane	0	0	0	0	0	0	0	0	0
2,3-dimethylbutane	0	0	0	0	0	0	0	0	0
2-methylpentane	0	0	0	0	0	0	0	0	0
3-methylpentane	0	0	0	0	0	0	0	0	0
DDD	0	0	0	0	0	0	0	0	0
DDE	0	0	0	0	0	0	0	0	0
benzyl alcohol	4	0	0	0	0	0	0	0	0
cis-2methylcyclohexanol	0	4	0	0	0	0	0	0	0
cyclohexylmethanol	0	0	4	0	0	0	0	0	0
m-cresol	0	0	0	4	0	0	0	0	0
n-hexanes	0	0	0	0	4	0	0	0	0
o-cresol	0	0	0	0	0	4	0	0	0
opDDT	0	0	0	0	0	0	4	0	0
p-cresol	0	0	0	0	0	0	0	4	0
pcb209	0	0	0	0	0	0	0	0	4
pcb29	0	0	0	0	0	0	0	0	0
pcb3	0	0	0	0	0	0	0	0	0
pcb52	0	0	0	0	0	0	0	0	0
pcb77	0	0	0	0	0	0	0	0	0
ppDDT	0	0	0	0	0	0	0	0	0
trans-2methylcyclohexano	0	0	0	0	0	0	0	0	0
Total	4	4	4	4	4	4	4	4	4

Jackknifed Classification Matrix (Contd.)

	pcb29	pcb3	pcb52	pcb77	ppDDT	trans-2methylcyclohexano	%correct
1-methylcyclohexanol	0	0	0	0	0	0	100
1-methylcyclopentane	0	0	0	0	0	0	100
2,3-dimethylbutane	0	0	0	0	0	0	100
2-methylpentane	0	0	0	0	0	0	100
3-methylpentane	0	0	0	0	0	0	100
DDD	0	0	0	0	0	0	100
DDE	0	0	0	0	0	0	100
benzyl alcohol	0	0	0	0	0	0	100
cis-2methylcyclohexanol	0	0	0	0	0	0	100
cyclohexylmethanol	0	0	0	0	0	0	100
m-cresol	0	0	0	0	0	0	100
n-hexanes	0	0	0	0	0	0	100
o-cresol	0	0	0	0	0	0	100
opDDT	0	0	0	0	0	0	100
p-cresol	0	0	0	0	0	0	100
pcb209	0	0	0	0	0	0	100
pcb29	4	0	0	0	0	0	100
pcb3	0	4	0	0	0	0	100
pcb52	0	0	4	0	0	0	100
pcb77	0	0	0	4	0	0	100
ppDDT	0	0	0	0	4	0	100
trans-2methylcyclohexano	0	0	0	0	0	4	100
Total	4	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.908	0.994	1.000
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Aromatic alcohols

Jackknifed Classification Matrix

	benzyl alcohol	m-cresol	o-cresol	p-cresol	%correct
benzyl alcohol	4	0	0	0	100
m-cresol	0	4	0	0	100
o-cresol	0	0	4	0	100
p-cresol	0	0	0	4	100
Total	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.859	0.950	1.000
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Pesticides

Jackknifed Classification Matrix

	DDD	DDE	opDDT	ppDDT	%correct
DDD	4	0	0	0	100
DDE	0	4	0	0	100
opDDT	0	0	4	0	100
ppDDT	0	0	0	4	100
Total	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.995	1.000	1.000
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Alkanes

Jackknifed Classification Matrix

	1-methylcyclopentane	2,3-dimethylbutane	2-methylpentane	3-methylpentane	n-hexanes	%correct
1-methylcyclopentane	4	0	0	0	0	100
2,3-dimethylbutane	0	4	0	0	0	100
2-methylpentane	0	0	4	0	0	100
3-methylpentane	0	0	0	4	0	100
n-hexanes	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.767	0.930	1.000
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Aliphatic alcohols

Jackknifed Classification Matrix

	1-methylcyclohexanol	cis-2methylcyclohexanol	cyclohexylmethanol	trans-2methylcyclohexanol	%correct
1-methylcyclohexanol	4	0	0	0	100
cis-2methylcyclohexanol	0	4	0	0	100
cyclohexylmethanol	0	0	4	0	100
trans-2methylcyclohexanol	0	0	0	4	100
Total	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.775	0.990	1.000
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PCBs

Jackknifed Classification Matrix

	pcb209	pcb29	pcb3	pcb52	pcb77	%correct
pcb209	4	0	0	0	0	100
pcb29	0	4	0	0	0	100
pcb3	0	0	4	0	0	100
pcb52	0	0	0	4	0	100
pcb77	0	0	0	0	4	100
Total	4	4	4	4	4	100

Cumulative Proportion of Total Dispersion

0.806	0.996	1.000
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1:1 binary mixtures of analytes 5-8

Jackknifed Classification Matrix

	BA-M	BA-O	BA-P	M-P	O-M	O-P	%correct
BA-M	2	0	0	0	0	2	50
BA-O	0	4	0	0	0	0	100
BA-P	1	0	3	0	0	0	75
M-P	0	0	0	4	0	0	100
O-M	0	0	0	0	4	0	100
O-P	1	0	0	0	0	3	75
Total	4	4	3	4	4	5	83

Cumulative Proportion of Total Dispersion

0.889	0.981	1.000
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SUMMARY FIGURES

SUMMARY FIGURES FOR FLUORESCENCE MODULATION

o-Cresol

m-Cresol

p-Cresol

DDD

DDE

o,p-DDT

p,p-DDT

n-Hexanes

2-Methylpentane

3-Methylpentane

2,3-Dimethylbutane

1-Methylcyclopentane

1-Methylcyclohexanol

cis-2-Methylcyclohexanol

trans-2-Methylcyclohexanol

PCB3

PCB29

PCB52

PCB77

PCB209

SUMMARY FIGURES LIMIT OF DETECTION

p,p-DDT – S1

p,p-DDT – S2

p,p-DDT – S3

o-Cresol – S1

Benzyl alcohol – S2

o-Cresol – S3

Cyclohexylmethanol – S1

Cyclohexylmethanol – S2

1-Methylcyclohexanol – S3

PCB77 – S1

PCB209 – S2

PCB209 – S3

2-Methylpentane – S1

3-Methylpentane – S2

1-Methylcyclopentane – S3

SUMMARY FIGURES ARRAYS

All Analytes

Aromatic alcohols

Pesticides

Alkanes

Alcohols

PCBs

1:1 binary mixtures of analytes **5-8**

NMR SPECTRA OF ALL NEW COMPOUNDS

Compound 2

^1H NMR

^{13}C NMR

COSY NMR

Compound 3

¹H NMR

SPECTROSCOPIC INVESTIGATIONS OF SENSORS **S1-S3**

ABSORPTION SPECTRA

UV-Visible Absorption Spectra of **S2** and **S3** (1 μ M) in DMSO measured at room temperature:

VARIATION OF FLUORESCENCE EMISSION OF SENSORS IN H₂O/DMSO MIXTURES

Fluorescence emission spectra of **S1**, **S2** and **S3** (at 1 μM concentration) in 80:20 (H_2O : DMSO) (black trace), 60:40 (H_2O : DMSO) (red trace), 40:60 (H_2O : DMSO) (blue trace), 20:80 (H_2O : DMSO) (purple trace), 0:100 (H_2O :DMSO) (green trace). ($\lambda_{\text{ex}} = 320 \text{ nm}$). All spectra were recorded at room temperature.

BENESI-HILDEBRAND PLOTS FOR NMR TITRATION

Analyte **5** (0.2 M in 0.8 mL D_2O) was titrated against 0 μL , 10 μL , 20 μL , 25 μL , 30 μL , 35 μL , 40 μL , 50 μL , 60 μL , 80 μL and 100 μL of the host (1 mg/mL dissolved in d_6 -DMSO) in a clean dry NMR tube. The volume was adjusted to 1.0 mL final volume with the addition of d_6 -DMSO. The ^1H -NMR spectra of the samples were recorded in 300 MHz Bruker AVANCE NMR Spectrometer at room temperature. The chemical shift of benzylic protons (highlighted in red in the figure below) were tracked, and the data was used to solve the Benesi-Hildebrand equation, below.

Benesi-Hildebrand Equation:

$$1/\Delta\delta = (1/K_a \Delta\delta_{\max})1/[H] + (1/\Delta\delta_{\max}) \quad (\text{Eq. S3})$$

Host	Equation	K_a (M^{-1})	$\Delta\delta_{\max}$ (ppm)
1	$y = 0.0045x + 162.97$	$3.6(0.1) \times 10^4$	0.0061
2	$y = 0.0024x + 116.62$	$4.8(0.5) \times 10^4$	0.0085
3	$y = 0.0007x + 173.27$	$24.9(0.5) \times 10^4$	0.0057

Benesi-Hildebrand plots for association constant calculations of analyte **5** with compounds **1**, **2** and **3** in 80:20 water-DMSO at room temperature. (H is the host; K_a is association constant; $\Delta\delta_{\max}$ is maximum peak shift at infinite host concentration $[H] = \infty$; $\Delta\delta$ is the peak shift at a given host concentration)

CHAPTER 2

Enhanced binding complex characterization of fluorophores by fluorescence spectral variation in cyclodextrin mixtures

ABSTRACT

Extensive literature has reported the properties of pyrene in β -cyclodextrin and γ -cyclodextrin, but little has been published on β -cyclodextrin derivatives (methyl- β -cyclodextrin and 2-hydroxypropyl- β -cyclodextrin) or mixtures of different cyclodextrins. This experiment focused on characterizing pyrene's interactions, as well as those pyrene-d10 and benzo[*a*]pyrene, within different cyclodextrin solutions using fluorescence spectroscopy. Vibronic bands within the fluorescence emission spectrum of pyrene reflected the polarity of the microenvironment around pyrene (as measured by Py values). Little change in the Py values was observed when pyrene was introduced to different concentrations of methyl- β -cyclodextrin and 2-hydroxypropyl- β -cyclodextrin. Benzo[*a*]pyrene had a large excimer peak when doped into γ - or β : γ solutions, which suggested that benzo[*a*]pyrene may be forming 2:1 complexes with the cyclodextrins. Deuterated pyrene (pyrene-d10) had a lower binding constant in β -cyclodextrin (compared to pyrene-h10), but a higher binding constant in γ -cyclodextrin (compared to pyrene-h10). Further studies should be conducted to determine why deuterium incorporation would produce a higher binding constant in the γ -cyclodextrin solutions.

INTRODUCTION

The hydrophobic cavity of cyclodextrin can be used as a binding site for a variety of environmentally important applications, such as molecular transportation¹⁻³,

extraction⁴⁻⁶, or the fluorescence enhancement of environmental toxicants^{7,8}. Guest binding to cyclodextrin can be driven by several different factors, including the displacement of water from the cyclodextrin cavity and an increase in the hydrophobic interactions between guest and host, as well as the low steric hindrance of the guest within the cyclodextrin cavity.⁹ Cyclodextrin cavity sizes vary and combining different cyclodextrins either through physically attaching them or through non-covalent mixing could increase the net energetic driving force, which in turn is likely to increase the guest binding affinity.¹⁰

Extensive research has been done on the binding of pyrene,¹¹ which is a polycyclic aromatic hydrocarbon (PAH), to cyclodextrin, but far less has been studied on other PAHs binding to cyclodextrin. Pyrene's fluorescence characteristics, such as its fluorescence lifetime and fluorescence emission spectrum,¹²⁻¹⁴ depend strongly on solvent polarity. In fact, within the emission spectrum of pyrene, band I (at ~373 nm) and band III (at ~383 nm), which are associated with different vibrational states of pyrene, change intensity depending on solvent interactions.¹²⁻¹⁵ The solvent polarity can be quantified in the pyrene fluorescence emission spectrum by dividing band I by band III (also referred to as the Py value or I/III value).¹²

Figure 1. Pyrene doped into 10 mM β -cyclodextrin in water. Band I and Band III represent fluorescence emissions from different relaxation pathways.

As the polarity of the solvent increases, the Py value is typically greater than 1. Accordingly,¹⁵ Py values can be used for the determination of solvent polarity due to the strong $S_0 \rightarrow S_2$ absorption between electronic states and the relaxation between $S_1 \rightarrow S_0$. Band I corresponds to the fluorescence relaxation between $S_1^{v=0} \rightarrow S_0^{v=0}$ and band III corresponds to the fluorescence relaxation between $S_1^{v=0} \rightarrow S_0^{v=1}$. The Py value has been found to be independent of the presence of oxygen.¹⁵ Py values have been used to calculate the binding constant of pyrene to a host as described in Equation 1.¹¹

$$\text{Equation 1. } \frac{[PCD]}{[P]_0} = \frac{Py_0 - Py_{CD}}{Py_0 - Py_X}$$

Pyrene is found to have a 2:1 CD:pyrene stoichiometry rather than a 1:1 complex when mixed with high concentrations of beta-cyclodextrin (β -CD).¹¹ A 1:1 complex is more favorable than 2:1 CD:pyrene complex for pyrene in gamma-cyclodextrin (γ -

CD) solutions, although at high concentrations of γ -CD, 2:2 complexation may be a more favorable state.^{11,16} The 2:1 complex may be favored due to the limited space in the β -CD cavity,^{11,9} which would decrease the polarity around pyrene (giving a Py value less than 1). Limited research has been conducted on PAH binding with β -CD derivatives, such as methyl- β -cyclodextrin (M β -CD) and 2-hydroxypropyl- β -cyclodextrin (2HPCD), as well as on mixtures of cyclodextrins. This study's goal is to explore the binding characteristics of pyrene and other PAHs to cyclodextrin and cyclodextrin mixtures.

METHOD

20 mM stock solutions of γ -CD, M β -CD, and 2HPCD were prepared as well as a 15 mM stock solution of β -CD (due to the low solubility of β -CD). These stock solutions were used to prepare the 10 mM solutions of the single component cyclodextrin mixtures (β -CD, γ -CD, M β -CD, and 2HPCD) and the mixed cyclodextrin solutions (β : γ -CD, β :M β -CD, β :2HPCD, γ :M β -CD, γ :2HPCD, M β :2HPCD). PAH stock solutions (pyrene and benzo[*a*]pyrene) were prepared at a concentration of 1 mg/mL in tetrahydrofuran (THF). 2.5 mL of the 10 mM cyclodextrin solutions was added to a cuvette and the fluorescence of the solution run (in quadruplicate) at the PAH excitation wavelength λ_p (335 nm for pyrene and 360 nm for benzo[*a*]pyrene) and collected from λ_p +10 nm to 710 nm. The absorption spectra of the solutions were also measured, from 180 nm to 900 nm. An aliquot of the selected PAH solution was doped into the cuvette at 0.5 μ L and the fluorescence and absorption spectra were run again. PAH solution was continually added until a total of 2 μ L of solution had been reached. The data was then extracted from the fluorescence and absorption

instruments and compiled into a worksheet. Each fluorescence spectrum was integrated with wavenumber as the X-axis, and the fluorescence spectra with pyrene as the analyte at the location of band I and band III were recorded as well as the fluorescence intensity at the bands. The Py value of each spectrum was determined by dividing band I by band III. The average Py value for each solution (from the quadruplicate data collection) was used for the polarity comparison. The spectra were also analyzed for the presence of excimer peaks and the excimer peak intensities were recorded and then integrated. Binding constants were calculated based on the Py values received and fitted with linear and nonlinear regression lines then compared to the binding constant calculated by using integrated fluorescence emissions.

Binding constants for 1:1 guest:cyclodextrin were calculated by using the Benesi-Hildebrand method.¹⁷ After integrating each spectra, the inverse integration was plotted against the inverse concentration of cyclodextrin in molarity. A best fitted line with an R^2 value of 0.95 or greater represents a 1:1 guest:cyclodextrin complex. The binding constant was calculated using the slope of the line and the integrated fluorescence emission of pyrene in pure water.

RESULTS

The fluorescence intensity of pyrene increased as the amount of pyrene added to the solutions increased; however, the Py value was consistent with small concentrations of pyrene (Table 1). Py values only changed significantly when the solvent polarity changed. At concentrations of β -cyclodextrin greater than 1 mM, the Py value was below 1, similar to what was found in the non-polar solutions (*n*-octane). Pure γ -cyclodextrin solutions showed Py values greater than 1 at all concentrations.

Table 1. Pyrene (1 mg/mL in THF) doped into different solvents. I/III ratios calculated from each fluorescence spectrum.

2.5 mL of solvent	Conc. (μ L)	Py value
DI water	0.5	1.94 ± 0.04
DI water	1	1.96 ± 0.05
β -CD 10 mM	0.5	0.96 ± 0.01
β -CD 10 mM	1	0.95 ± 0.01
β : γ CD (5:5)	0.5	0.98 ± 0.01
β : γ CD (5:5)	1	0.96 ± 0.01
γ -CD 10mM	0.5	1.66 ± 0.05
γ -CD 10mM	1	1.67 ± 0.03
<i>n</i> -octane	1	0.74 ± 0.04

Similar trends were seen between the monomer emission (345 – 455 nm) and excimer emission (456 – 600 nm) integration. High intensities of monomer were seen in high concentrations of β -cyclodextrin, but the monomer intensity drastically decreases when β -cyclodextrin concentrations drop below 1 mM. A general increase of the excimer emission is seen in pure γ -cyclodextrin, but excimer emission is higher in the β : γ mixture (from cyclodextrin ratios above No significant excimer peaks were seen with pure β -cyclodextrin solutions.

Figure 2. A. Monomer emission (345 – 455 nm) and B. Excimer emission (456 – 600 nm) intensity with increasing concentration of γ -cyclodextrin.

Figure 3. B-H plot for the binding of pyrene-h10 to β -cyclodextrin. The best fit line $y = 5E-10X + 1E-6$ with an R^2 value of 0.9605 and calculated binding constant: $K_a = 2000 M^{-1}$.

Figure 4. B-H plot for the binding of pyrene-h10 to γ -cyclodextrin. The best fit line $y = 2E-9x + 4E-6$ with an R^2 value of 0.9335 and calculated binding constant: $K_a = 2000 M^{-1}$.

Figure 5. B-H plot for the binding of pyrene-d10 to β -cyclodextrin. The best fit line $y = (6\text{E-}10)x + 2\text{E-}6$ with an R^2 value of 0.9784 and calculated binding constant: $K_a = 3333 \text{ M}^{-1}$.

Figure 6. B-H plot for the binding of pyrene-d10 to γ -cyclodextrin. The best fit line $y = (2\text{E-}9)x + (2\text{E-}6)$ with an R^2 value of 0.9735 and calculated binding constant: $K_a = 1000 \text{ M}^{-1}$.

Figure 7. Benzo[a]pyrene (1mg/mL) solution doped in 2.5 mL of A. water, B. 10 mM β -cyclodextrin, C. 10 mM γ -cyclodextrin, D. 5 mM β -cyclodextrin: 5 mM γ -cyclodextrin.

Table 2. Benzo[a]pyrene Fluorescence (Fl) Ratio (integration of benzo[a]pyrene in host solution/integration of benzo[a]pyrene in water) and Excimer ratio (integration of excimer peak of benzo[a]pyrene/integration of the monomer peak of benzo[a]pyrene).

Host	Fl Ratio	Excimer Ratio
n-octane		0.31 ± 0.00
DI water		2.08 ± 0.11
β -CD	20.24 ± 0.42	0.23 ± 0.00
1:1 β -CD: γ -CD	9.23 ± 0.04	6.32 ± 0.05
γ CD	8.76 ± 0.05	15.11 ± 0.11

DISCUSSION

Pyrene is sterically constrained as to how it can occupy the cavity of cyclodextrin. β -cyclodextrin has a cavity size of $7.8 \text{ \AA}$,¹⁸ which doesn't allow pyrene to fit along its z-axis (Figure 8) and restricts it to the y-axis,¹⁹ and γ -cyclodextrin has a cavity size of $9.5 \text{ \AA}$,¹⁸ which is large enough to fit pyrene along the z- or y-axis.¹⁹ The steric limitations of pyrene fitting into β -cyclodextrin would leave pyrene partially exposed to the polar solvent. However, as the concentration of β -cyclodextrin increases, there is evidence that it moves from a 1:1 pyrene:cyclodextrin environment to a 1:2 pyrene:cyclodextrin environment, which would encapsulate pyrene and represent a more non-polar microenvironment (seen by the shift in the I/III ratio from > 1 to < 1). Since γ -cyclodextrin has a larger cavity size, our results suggest that water may also occupy the cavity along with the pyrene, maintaining a more polar microenvironment (I/III is still > 1 at all concentrations of γ -cyclodextrin). As γ -cyclodextrin concentration increases above 1 mM, the excimer peak increased (470 nm), which indicates the likelihood of a 2:2 pyrene:cyclodextrin complex.¹⁶ There is a continual increase in the excimer/monomer ratio as the γ -cyclodextrin concentration increases, however when mixed with β -cyclodextrin, there is a ~30% increase in the excimer/monomer ratio (table excimer). This could be due to the formation of 1:1:1 pyrene: β -cyclodextrin: γ -cyclodextrin, but the different number of cyclodextrin

monomers in between γ and β -cyclodextrin (β – 7 glucose units, γ – 8 glucose units) may sterically restrict this complexation.

Figure 8. One of pyrene's 6 Kekulé structures with the vertical axis defined as the z-axis and the horizontal axis defined as the y-axis.²⁰

The microenvironment around pyrene in the β : γ mixed cyclodextrin solutions transitions from a polar environment at low concentrations of β -cyclodextrin to a non-polar environment above 1-2 mM β -cyclodextrin. This suggests that there is no 1:2 pyrene: β -cyclodextrin below 1 mM of β -cyclodextrin. The increase in I/I_0 ratio may be attributed to the addition of both pyrene: γ -cyclodextrin and pyrene: β -cyclodextrin fluorescence signals. Further investigation using life-time studies could determine whether there is a new conformation between two different cyclodextrins and the pyrene.

Methyl- β -cyclodextrin and 2-hydroxypropyl- β -cyclodextrin both I/III band ratios that were greater than 1 and the absence of any notable excimer peak (Figure 8). Even as the concentration of each β -cyclodextrin derivative increases, there is no change in the Py values. This suggests that pyrene maintains a polar

microenvironment when occupying the cavity of methyl- β -cyclodextrin or 2-hydroxypropyl- β -cyclodextrin. No significant change in the Py value was seen when either β -cyclodextrin derivative was mixed with β -cyclodextrin (Table S1).

Figure 9. 1 μ L of pyrene (1mg/mL) doped into 2.5 mL of A. methyl- β -cyclodextrin and B. 2-hydroxypropyl- β -cyclodextrin.

The binding constant of pyrene-d10 to β -cyclodextrin increased compared to pyrene-h10 with β -cyclodextrin, however, the opposite was seen with γ -cyclodextrin. Previous studies have reported that deuterated guest molecules decrease in binding with cyclodextrin host.²¹ Decrease in the binding constant of deuterated pyrene may be caused by the change in enthalpy with the addition of heavy hydrogen.

Preliminary results for benzo[*a*]pyrene and mixed cyclodextrins suggest that there is a significant increase in the fluorescence signal with the addition of cyclodextrin, but the excimer of benzo[*a*]pyrene is only present when γ -cyclodextrin is one of the host molecules or in the complete absence of a host molecule. At 1:1 β -cyclodextrin: γ -cyclodextrin mixture, the monomer signal increased significantly from benzo[*a*]pyrene in a pure water solution or a pure γ -cyclodextrin solution, however, the excimer signal decrease in the mixture solution compared to the excimer signal

from a pure γ -cyclodextrin solution. This suggests that benzo[*a*]pyrene may be occupying both β -cyclodextrin and γ -cyclodextrin. Further studies on whether the excimer is due to a 2:1 benzo[*a*]pyrene:cyclodextrin complex or a 2:2 benzo[*a*]pyrene:cyclodextrin complex would involve increase the range of concentrations of the cyclodextrin solutions to see if the excimer peak is eliminated at lower concentrations (similar to the pyrene and cyclodextrin complexation).

CONCLUSION

Due to steric hindrance, pyrene's microenvironment varies greatly between cyclodextrins cavities. When the concentration of cyclodextrin increase above 1 mM of β -cyclodextrin or γ -cyclodextrin, the pyrene:cyclodextrin complexation changes from 1:1 to either a 2:1 or a 2:2 complexation. Our results were not able to determine whether there is a 1:1:1 pyrene: β -cyclodextrin: γ -cyclodextrin complex forming, further fluorescence life-time studies could help determine if a 1:1:1 complex is forming. Deuterated pyrene showed an increase in binding to β -cyclodextrin, which does not support the literature reported on the binding of deuterated analytes. Although benzo[*a*]pyrene does not have a change in its band ratios with a change in solvent polarity, the change in the excimer peak ratios is evidence that benzo[*a*]pyrene has a higher affinity for γ -cyclodextrin compared to β -cyclodextrin, which is the opposite that we see with pyrene.

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Enhanced binding complex characterization of fluorophores by fluorescence spectral variation in cyclodextrin mixtures

Table S1. Py values for mixed cyclodextrins containing 1 mM β -cyclodextrin and 9 mM of 2-hydroxypropyl- β -cyclodextrin, methyl- β -cyclodextrin, or γ -cyclodextrin.

	CD ratio	Py (I/III)
β CD:2HP β CD	1:9	1.147
β CD:M β CD	1:9	1.171
β CD: γ CD	1:9	1.111

Table S2. Excimer/monomer ratio calculations based on the integrated values found for the monomer and the excimer emissions.

β - γ (mM:mM)	Monomer (345-455 nm)	Excimer (456-600 nm)	E/M		γ CD (mM)	Monomer (345-455 nm)	Excimer (456-600 nm)	E/M
9:1	8.64E+05	1.74E+04	0.020		0.1	1.28E+05	1.41E+04	0.111
8:2	8.70E+05	1.77E+04	0.020		0.15	1.44E+05	1.47E+04	0.102
7:3	8.97E+05	1.78E+04	0.020		0.2	1.52E+05	1.98E+04	0.131
6:4	8.64E+05	1.76E+04	0.020		0.4	1.08E+05	1.07E+04	0.099
5:5	8.56E+05	1.74E+04	0.020		0.6	1.19E+05	1.20E+04	0.101
4:6	8.55E+05	1.75E+04	0.020		0.8	1.27E+05	1.35E+04	0.106
3:7	8.25E+05	1.78E+04	0.022		1	1.42E+05	1.49E+04	0.105
2:8	8.71E+05	1.91E+04	0.022		2	1.77E+05	2.31E+04	0.131
1:9	7.39E+05	2.88E+04	0.039		4	1.87E+05	2.89E+04	0.155
0.8:9.2	5.96E+05	5.12E+04	0.086		6	2.18E+05	4.18E+04	0.191
0.4:9.6	4.48E+05	5.52E+04	0.123		8	2.37E+05	4.40E+04	0.186
0.2:9.8	3.64E+05	7.05E+04	0.194		10	2.35E+05	4.37E+04	0.186
0.1:9.9	3.44E+05	8.03E+04	0.234		11	2.65E+05	6.12E+04	0.231
0.05:9.95	3.13E+05	7.55E+04	0.242		12	2.45E+05	5.53E+04	0.226
0.01:9.99	3.03E+05	7.39E+04	0.244		14	2.39E+05	5.36E+04	0.225
0.005:9.995	3.12E+05	8.29E+04	0.266		16	2.57E+05	6.00E+04	0.234
					18	2.74E+05	6.46E+04	0.236
					20	2.68E+05	6.87E+04	0.257

Table S3. Py values calculated for γ -, β -, and β : γ cyclodextrin concentration used during the course of this experiment.

β - γ	β - γ			γ CD	β CD
mM:mM	I/III		mM	I/III	I/III
0.005:9.995	1.60		0.005	1.89	1.93
0.01:9.99	1.59		0.01	1.92	1.89
0.05:9.95	1.59		0.02	1.91	1.87
0.1:9.9	1.55		0.04	1.87	1.79
0.2:9.8	1.48		0.08	1.86	1.63
0.4:9.6	1.31		0.1	1.86	1.50
0.8:9.2	1.17		0.15	1.89	1.36
1:9	1.06		0.2	1.86	1.30
2:8	1.11		0.4	1.83	1.11
3:7	0.97		0.6	1.79	1.07
4:6	0.97		0.8	1.77	1.04
5:5	0.98		1	1.75	1.00
6:4	0.98		2	1.73	0.99
7:3	0.98		4	1.70	0.98
8:2	0.97		6	1.66	0.96
9:1	0.95		8	1.64	0.97
			10	1.60	0.95
			11	1.55	
			12	1.43	
			14	1.55	
			16	1.52	
			18	1.55	
			20	1.56

Figure S1. High concentrations of 2HP β CD 1 mM - 10 mM. Equation: $y = 2E-8x + 7E-7$ and $R^2: 0.9652$

Figure S2. Py values for low concentrations of β -cyclodextrin and low concentrations of β -cyclodextrin in mixed cyclodextrin solution.

Table S4. Integration ratios between the integration of the fluorescence emissions with the host ($I_{w/Host}$) / the integration of the fluorescence emissions of pyrene in water (I_{water}), $I_{w/Host}/I_{water}$

	$\beta:\gamma$			γ CD	β CD
mM:mM	I/I0		mM	I/I0	I/I0
0.005:9.995	3.08		0.005	1.10	1.08
0.01:9.99	2.94		0.01	1.13	1.13
0.05:9.95	3.03		0.02	1.09	1.13
0.1:9.9	3.31		0.04	1.12	1.24
0.2:9.8	3.39		0.08	0.93	1.52
0.4:9.6	3.94		0.1	1.11	1.87
0.8:9.2	5.06		0.15	1.24	2.54
1:9	5.74		0.2	1.34	2.23
2:8	6.96		0.4	0.92	3.27
3:7	6.59		0.6	1.03	3.44
4:6	6.82		0.8	1.10	3.85
5:5	6.82		1	1.23	4.46
6:4	6.89		2	1.56	5.69
7:3	7.15		4	1.69	5.28
8:2	6.94		6	2.03	6.48
9:1	6.89		8	2.19	6.94
			10	2.18	5.65
			11	2.55	
			12	2.34	
			14	2.28	
			16	2.48	
			18	2.65	
			20	2.63	