Electronic Supplementary Material (ESI) for New Journal of Chemistry. This journal is © The Royal Society of Chemistry and the Centre National de la Recherche Scientifique 2015

Electronic Supporting Information for

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

Dana J. DiScenza and Mindy Levine

# TABLE OF CONTENTS

Materials and Methods	S3
Analyte Details	S4
Synthesis of Fluorophore 6	S5
Experimental Details for Fluorescence Modulation Experiments	S7
Experimental Details for Limit of Detection Experiments	S8
Experimental Details for Fluorescence Array Experiments	S9
Experimental Details for <sup>1</sup> H NMR Titration Experiments	S10
Summary Tables	S11
Summary Table of Fluorescence Modulation Experiments	S11
Summary Table of Limit of Detection Experiments	S12
Summary Table of Fluorescence Array Experiments	S13
Summary Figures	S17
Summary Figures of Fluorescence Modulation Experiments	S17
Summary Figures of Limit of Detection Experiments	S22
Summary Figures of Fluorescence Array Experiments	S24

## 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



Final Analyte Concentrations:

Analyte Number	Amount Added	Final Concentration
1	20 μL	14.1 μM
2	20 μL	18.8 μM
3	20 µL	20.6 µM
4	20 µL	26.4 µM
5	20 μL	106 µM

Final Fluorophore Concentrations:

Fluorophore Number	Amount Added	<b>Final Concentration</b>
6	100 µL	9.5 μM
7	100 μL	8.1 μM
8	100 µL	11 μM

#### **SYNTHESIS OF FLUOROPHORE 6**

#### Synthesis of BODIPY (compound 6):

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

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:**



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 vellow. 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 literaturereported 24% yield).

#### **Reaction 2:**



<u>Procedure</u>: 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:**



<u>Procedure</u>: 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  $\mu$ 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  $\mu$ 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:

Fluorophore Ratio =  $(Fl_{alcohol})/Fl_{blank}$ 

(Equation 1)

where  $Fl_{alcohol}$  is the fluorescence emission of the fluorophore in the presence of the alcohol, and  $Fl_{blank}$  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:

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  $\gamma$ -cyclodextrin in phosphate-buffered saline (PBS) was measured into a cuvette and 100  $\mu$ 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  $\mu$ 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  $\mu$ L of analyte, 60  $\mu$ L of analyte, 80  $\mu$ L of analyte, and 100  $\mu$ 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  $\mu$ 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  $\gamma$ -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  $\mu$ M.

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  $\mu$ M.

## 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 <sup>1</sup>H NMR TITRATION EXPERIMENTS

A solution of 10 mM  $\beta$ -CD was prepared in deuterium oxide. In an NMR tube, the  $\beta$ -CD and varying concentrations (0-15 mM) of analytes were mixed. The <sup>1</sup>H NMR spectrum was run for each respective concentration of analyte in 10 mM  $\beta$ -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:

## $K_a = \Delta \delta / (\Delta \delta tot - \Delta \delta[G])$

The average K<sub>a</sub> value was determined for each analyte and fluorophore.

# SUMMARY TABLES FOR BINDING EXPERIMENTS

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

a Indicates nonsensical values for binding constants

# SUMMARY TABLES

Analyte	Fluorophore	α-CD	β-CD	Me-β-CD	2-HPCD	PBS
1	6	$1.12 \pm 0.00$	$1.15 \pm 0.01$	$1.01 \pm 0.01$	$1.10\pm0.01$	$1.22 \pm 0.02$
1	7	$1.09\pm0.00$	$1.00\pm0.00$	$1.00\pm0.00$	$1.02\pm0.00$	$1.00\pm0.00$
1	8	$1.21 \pm 0.01$	$1.01 \pm 0.03$	$0.97\pm0.01$	$0.88\pm0.01$	$1.22 \pm 0.01$
2	6	$1.15 \pm 0.02$	$1.15 \pm 0.01$	$0.97\pm0.01$	$1.14\pm0.02$	$1.33\pm0.02$
2	7	$0.98\pm0.00$	$1.00 \pm 0.01$	$0.99\pm0.01$	$0.97\pm0.00$	$1.00\pm0.00$
2	8	$1.07\pm0.02$	$1.03\pm0.04$	$0.87\pm0.02$	$0.94\pm0.01$	$1.10\pm0.00$
3	6	$1.30\pm0.04$	$1.24 \pm 0.02$	$0.96\pm0.01$	$1.18\pm0.02$	$1.27\pm0.01$
3	7	$1.08\pm0.02$	$0.96\pm0.02$	$1.04\pm0.00$	$0.99\pm0.01$	$0.88\pm0.00$
3	8	$1.08\pm0.03$	$1.24\pm0.14$	$0.94\pm0.01$	$0.88\pm0.00$	$1.23\pm0.01$
4	6	$1.26\pm0.02$	$1.15\pm0.01$	$0.99\pm0.01$	$1.08\pm0.01$	$1.34\pm0.01$
4	7	$1.28 \pm 0.01$	$1.05 \pm 0.05$	$1.03 \pm 0.02$	$0.99\pm0.00$	$1.09\pm0.03$
4	8	$1.21 \pm 0.01$	$1.08 \pm 0.13$	$0.83\pm0.02$	$0.94\pm0.00$	$1.23\pm0.00$
5	6	$1.05\pm0.02$	$1.06 \pm 0.02$	$0.99\pm0.00$	$1.07\pm0.01$	$1.20 \pm 0.05$
5	7	$0.98\pm0.00$	$0.98\pm0.00$	$0.98\pm0.00$	$0.99\pm0.00$	$0.98\pm0.00$
5	8	$1.07 \pm 0.01$	$0.88\pm0.01$	$0.98\pm0.00$	$0.97\pm0.01$	$0.99\pm0.01$

# SUMMARY TABLE OF FLUORESCENCE MODULATION EXPERIMENTS

Analyte	Fluorophore	CD	LOD (µM)	LOQ (µM)	Equation	$\mathbf{R}^2$
1	6	β-CD	82.4	162.3	y = 126651x + 4E7	0.9977
1	6	PBS	а	а	y = -125824x + 3E7	0.8856
2	6	β-CD	а	а	y= -137716x + 6E7	0.9916
2	6	PBS	а	а	y = 122235x + 2E7	0.8259
3	6	β-CD	31.3	53.3	y = 807091x + 4E7	0.9906
3	6	PBS	а	а	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

# SUMMARY TABLE OF LIMIT OF DETECTION EXPERIMENTS

a = nonsensical values were obtained

# SUMMARY TABLES OF FLUORESCENCE ARRAY EXPERIMENTS

## With α-CD:

## **Jackknifed Classification Matrix**

	ACDHeptachlor	ACDLindane	ACDMirex	ACDTHF	ACDcis-chlordan-	%correct
					е	
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**

|--|

## With β-CD:

## **Jackknifed Classification Matrix**

	BCDHeptachlor	BCDLindane	BCDMirex	BCDTHF	BCDcis-chlordan-	%correct
					е	
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

# With Me-β-CD:

#### **Jackknifed Classification Matrix**

	MeBCDHeptachlor	MeBCDLindane	MeBCDMirex	MeBCDTHF	MeBCDcis-chlord-	%correct
					ane	
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

With 2-HPCD:

#### Jackknifed Classification Matrix

	2HPCDHeptachlor	2HPCDLindane	2HPCDMirex	2HPCDTHF	2HPCDcis-chlord-	%correct
					ane	
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
-------------------

## With PBS:

#### **Jackknifed Classification Matrix**

	PBSHeptachlor	PBSLindane	PBSMirex	PBSTHF	PBScis-chlordan-	%correct
					е	
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

#### With all CD's:

#### Classification Matrix (Cases in row categories classified into columns) (Contd.)

	MeBCDMirex	MeBCDTHF	MeBCDcis-chlord-	PBSHeptachlor	PBSLindane	PBSMirex	PBSTHF	PBScis-chlordan-	%correct
			ane					е	
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

#### Jackknifed Classification Matrix (Contd.)

	ACDcis-chlordan-	BCDHeptachlor	BCDLindane	BCDMirex	BCDTHF	BCDcis-chlordan-	MeBCDHeptachlor	MeBCDLindane
	е					е		
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-chlord-	PBSHeptachlor	PBSLindane	PBSMirex	PBSTHF	PBScis-chlordan-	%correct
			ane					е	
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

|--|

## SUMMARY FIGURES SUMMARY FIGURES FOR ALL FLUORESCENCE MODULATION EXPERIMENTS Analyte 1 – Fluorophore 6



Analyte 1 – Fluorophore 7



Analyte 1 – Fluorophore 8







Analyte 2 – Fluorophore 7









ngth (nm)

Analyte 3 – Fluorophore 6



600

0.0



Analyte 3 – Fluorophore 8







Analyte 4 – Fluorophore 7







Analyte **5** – Fluorophore **6** 



Analyte **5** – Fluorophore **7** 



Analyte 5 – Fluorophore 8



# SUMMARY FIGURES FOR ALL LOD EXPERIMENTS:



Analyte 1 – Fluorophore 6– PBS



Analyte 2 – Fluorophore 6 – $\beta$ -CD



Analyte 2 – Fluorophore 6– PBS





Analyte **3** – Fluorophore **6**– PBS



Analyte 4 – Fluorophore 6– $\beta$ -CD



Analyte 4 - Fluorophore 6 - PBS



# SUMMARY FIGURES FOR ALL ARRAY EXPERIMENTS: With α-CD:





#### With 2-HPCD:

