Electronic Supplementary Information for

Efficient detection of polycyclic aromatic hydrocarbons and polychlorinated biphenyls via three-component energy transfer

Nicole Serio, Kayla Miller, and Mindy Levine

University of Rhode Island, Kingston, RI 02881; mlevine@chm.uri.edu

TABLE OF CONTENTS

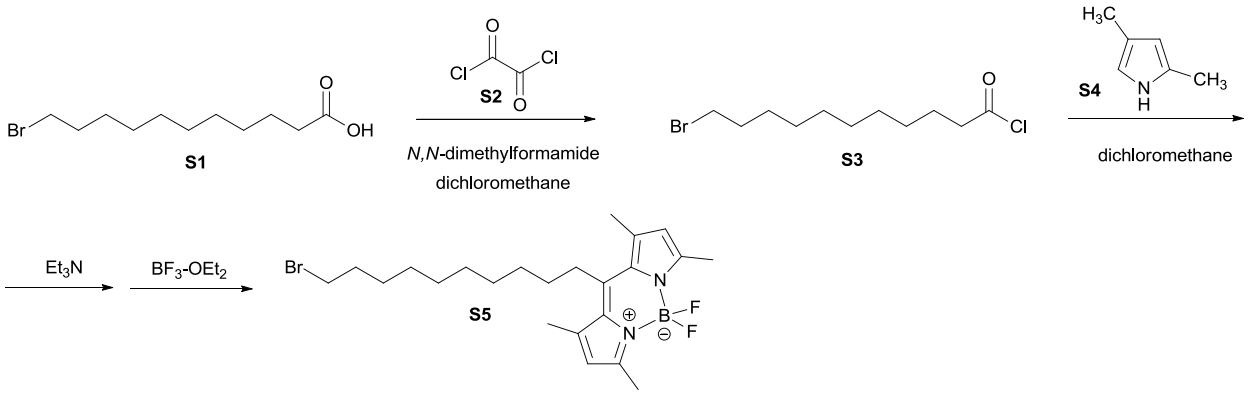
MATERIALS AND METHODS

All chemicals were purchased from Sigma-Aldrich Chemical Company and used as received, unless otherwise noted. ^{1}H 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-5301PC spectrophotofluorimeter.

SYNTHESES OF FLUOROPHORES

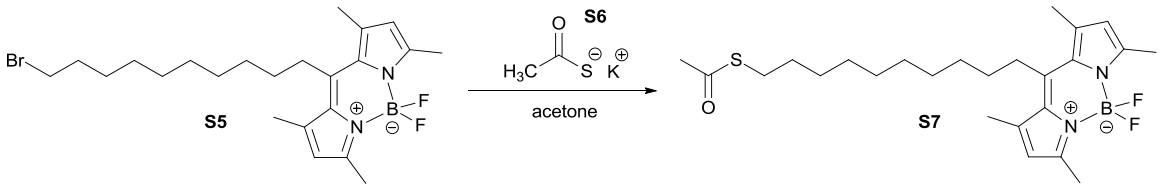
The synthesis of BODIPY 9 was performed according to literature procedures:[S1]

Reaction 1:



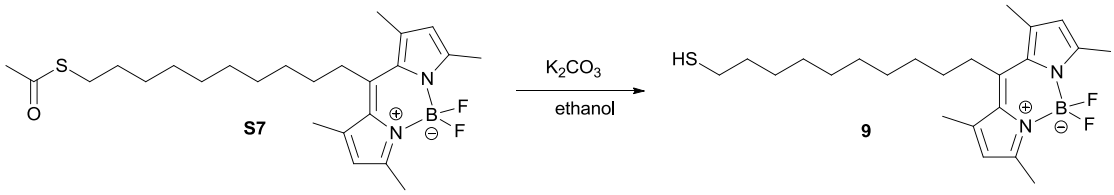
Procedure: 2.0 grams of 11-bromoundecanoic acid **S1** (7.54 mmol, 1.0 eq.) was combined with 2 drops of *N*,*N*-dimethylformamide in 40 mL of dichloromethane. 1.0 gram of oxalyl chloride **S2** (7.88 mmol, 1.05 eq.) was dissolved in 5.0 mL of dichloromethane and added dropwise. The reaction mixture 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 ^{o}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 ^{o}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 ^{o}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:



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).

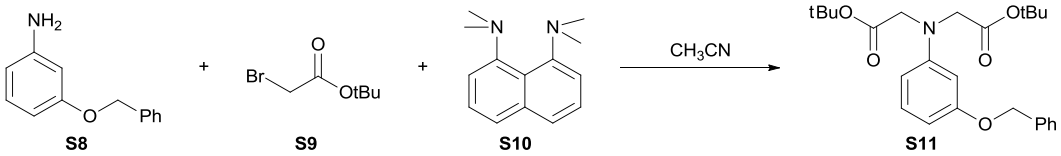
Reaction 3:



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 ^{o}C. The reaction mixture was stirred under nitrogen for 4 hours at 30 ^{o}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 **9** in 76% yield (674 mg).

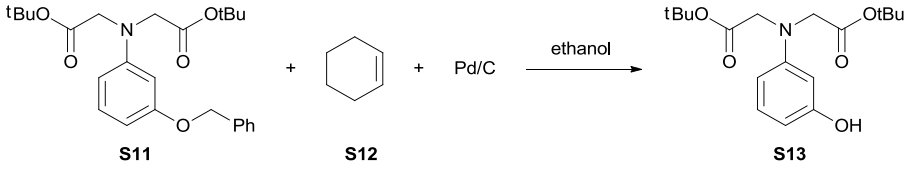
The synthesis of squaraine 10 was performed according to literature procedures:[S2]

Reaction 1:



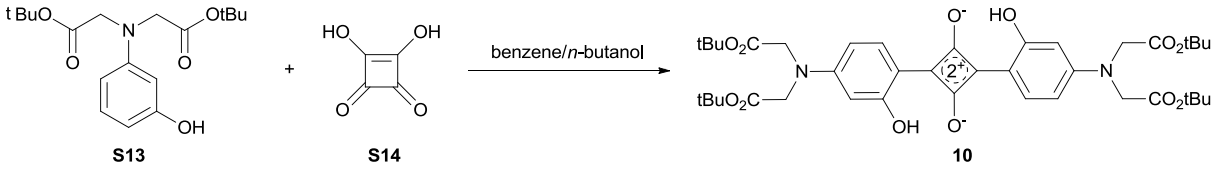
Procedure: Compound **S8** (0.912 g, 4.58 mmol, 1.0 eq.) and compound **S10** (2.16 g, 10.08 mmol, 2.2 eq.) were dissolved in 15 mL acetonitrile. Compound **S9** (1.42 mL, 9.62 mmol, 2.1 eq.) was added, and the reaction mixture was heated to reflux for 20 hours, at which time additional portions of compounds **S9** and **S10** were added and the reaction mixture was heated to reflux for another five hours. The reaction mixture was then cooled to room temperature. The solids were filtered and washed with ethyl acetate. The filtrate was then washed with brine, dried over magnesium sulfate, filtered and concentrated. Flash chromatography with 10% ethyl acetate in hexanes yielded compound **S11** in 72% yield (1.41 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.426-7.309 (m, 5 H), 7.105 (t, J = 8.4 Hz, 1 H), 6.39 (dd, J = 2 Hz, J = 8 Hz, 1 H), 6.249-6.202 (m, 2 H), 5.019 (s, 2 H), 3.989 (s, 4 H), 1.480 (s, 18 H).

Reaction 2:



Procedure: Compound **S11** (0.877 mmol, 1.0 eq., 375 mg) was dissolved in 37 mL of ethanol. 10% palladium on carbon (516 mg) was added, followed by cyclohexene (compound **S12**, 102 mmol, 116 eq., 10.32 mL). The reaction mixture was heated to reflux for two hours. The reaction mixture was then cooled to room temperature, and filtered through celite to remove the palladium. The filtrate was concentrated, and purified by flash chromatography (10% ethyl acetate in hexanes) to yield compound **S13** (296 mg, quantitative yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.038 (t, J = 8.4 Hz, 1 H), 6.237-6.141 (m, 2 H), 6.087 (t, J = 2.4 Hz, 1 H), 3.977 (s, 4 H), 1.438 (s, 18 H).

Reaction 3:



S13 S14 10

Procedure: Compound **S13** (0.877 mmol, 2.0 eq, 296 mg) was dissolved in 8 mL of benzene and 8 mL of *n*-butanol. Compound **S14** (0.439 mmol, 1.0 eq, 50 mg) was added, and the reaction mixture was equipped with a Dean-Stark trap and condenser, and heated to reflux for 24 hours. After 24 hours, the reaction mixture was cooled to room temperature and concentrated to yield compound **10** directly. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07 (d, J = 9.2 Hz, 0.1 H), 7.92 (d, J = 9.2 Hz, 0.9 H), 6.24 (d, J = 9.2 Hz, 2 H), 6.069 (s, 2 H), 3.970 (s, 8 H), 1.444 (s, 36 H). ESI-MS: 753.33 (m), 775.31 ($m+Na^+$). FTIR (KBr pellet, cm^{-1}): 3439 (m), 1738 (m), 1613 (s), 1383 (m), 1147 (s), 810 (m).

CONTROL EXPERIMENTS

These experiments were designed to determine the emission of the fluorophores from excitation at various wavelengths (in the absence of the analyte) and compare it to the emission of fluorophores at the same wavelengths in the presence of the analyte. This will determine whether an observed "energy transfer" peak may simply be a result of exciting the fluorophore at a wavelength where it has non-zero absorbance. These experiments were conducted as follows:

(a) The fluorophore was mixed with γ-cyclodextrin and excited at the excitation wavelength of the analyte (but in the absence of any analyte); and

(b) the fluorophore and analyte were both mixed in γ-cyclodextrin and excited at analyte excitation wavelength.

The fluorophore emission that resulted from excitation at the analyte wavelength in the absence of the analyte was compared to the fluorophore emission from excitation at the analyte wavelength in the presence of the analyte. The ratio of these two emissions, shown as "ratio of fluorophore emissions" in the tables below, is defined as:

Fluorophore emission via low wavelength excitation in the absence of an analyte/ fluorophore emission via low wavelength excitation in the presence of the analyte.

This was used to determine what fraction of that peak was a result of legitimate energy transfer rather than simple excitation of the fluorophore at a wavelength where it has non-zero absorbance.

All of these experiments were done with 1.5 nm excitation slit width and 1.5 nm emission slit width.

Anthracene (**1**) – Rhodamine (**8**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	0.99
2 mM	1.01
3 mM	0.99
4 mM	0.99
5 mM	1.01
6 mM	1.05
7 mM	1.00
8 mM	0.99
9 mM	1.09
10 mM	0.99

Anthracene (**1**) – BODIPY (**9**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	1.08
2 mM	1.05
3 mM	1.05
4 mM	1.04
5 mM	1.06
6 mM	1.05
7 mM	0.94
8 mM	1.06
9 mM	1.09
10 mM	0.98

Pyrene (**2**) – Rhodamine (**8**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	0.27
2 mM	0.27
3 mM	0.20
4 mM	0.27
5 mM	0.30
6 mM	0.52
7 mM	0.27
8 mM	0.32
9 mM	0.39
10 mM	0.20

Pyrene (**2**) – BODIPY (**9**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	0.91
2 mM	0.48
3 mM	0.33
4 mM	0.34
5 mM	0.43
6 mM	0.49
7 mM	0.42
8 mM	0.48
9 mM	0.43
10 mM	0.27

Benzo[*a*]pyrene (**3**) – Rhodamine (**8**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	0.24
2 mM	0.12
3 mM	0.09
4 mM	0.09
5 mM	0.13
6 mM	0.11
7 mM	0.06
8 mM	0.12
9 mM	0.16
10 mM	0.05

Benzo[*a*]pyrene (**3**) – BODIPY (**9**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	0.53
2 mM	0.21
3 mM	0.17
4 mM	0.16
5 mM	0.16
6 mM	0.15
7 mM	0.18
8 mM	0.15
9 mM	0.13
10 mM	0.09

Phenanthrene (**4**) – Rhodamine (**8**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	2.02
2 mM	2.20
3 mM	1.56
4 mM	1.78
5 mM	1.37
6 mM	2.08
7 mM	1.09
8 mM	2.01
9 mM	2.20
10 mM	1.48

Phenanthrene (**4**) – BODIPY (**9**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	1.26
2 mM	1.47
3 mM	1.06
4 mM	1.23
5 mM	1.10
6 mM	1.34
7 mM	1.02
8 mM	1.09
9 mM	0.99
10 mM	0.95

Fluorene (**5**) – Rhodamine (**8**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	1.76
2 mM	1.65
3 mM	1.60
4 mM	1.72
5 mM	1.66
6 mM	1.41
7 mM	1.04
8 mM	1.57
9 mM	2.46
10 mM	1.73

Fluorene (**5**) – BODIPY (**9**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	0.71
2 mM	0.73
3 mM	0.67
4 mM	0.71
5 mM	0.63
6 mM	0.63
7 mM	0.57
8 mM	0.61
9 mM	0.44
10 mM	0.60

4,4’-dichlorobiphenyl (**6**) – Rhodamine (**8**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	1.18
2 mM	1.07
3 mM	1.09
4 mM	1.14
5 mM	1.19
6 mM	1.02
7 mM	0.81
8 mM	1.14
9 mM	1.28
10 mM	1.10

4,4’-dichlorobiphenyl (**6**) – BODIPY (**9**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	1.10
2 mM	1.01
3 mM	1.04
4 mM	1.03
5 mM	1.05
6 mM	0.99
7 mM	1.02
8 mM	1.06
9 mM	0.98
10 mM	1.05

PCB29 (**7**) – Rhodamine (**8**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	1.13
2 mM	1.12
3 mM	1.11
4 mM	1.22
5 mM	1.19
6 mM	0.99
7 mM	1.02
8 mM	1.19
9 mM	1.32
10 mM	1.11

PCB29 (**7**) – BODIPY (**9**)

[γ-cyclodextrin]	Ratio of fluorophore emission
1 mM	1.13
2 mM	1.11
3 mM	1.05
4 mM	1.04
5 mM	1.10
6 mM	1.05
7 mM	1.01
8 mM	1.07
9 mM	1.09
10 mM	1.05

ALL ANALYTES WITH SQUARAINE (10) AT 10 MM γ-CYCLODEXTRIN:

Based on these results, we can divide the analyte-fluorophore pairs into three categories:

(a) Fluorophore emission ratios close to 1. These indicate that there is no significant interaction between the analyte and the fluorophore, and that the fluorophore peak from excitation at the analyte wavelength is merely due to the fluorophore absorbance at that wavelength. Pairs that fall into this category:

Anthracene (**1**) – Rhodamine (**8**)
Anthracene (**1**) – BODIPY (**9**)
Phenanthrene (**4**) – BODIPY (**9**)
4,4'-dichlorobiphenyl (**6**) – Rhodamine (**8**)
4,4'-dichlorobiphenyl (**6**) – BODIPY (**9**)
PCB29 (**7**) – Rhodamine (**8**)
PCB29 (**7**) – BODIPY (**9**)

(b) Fluorophore emission ratios higher than 1.

In these cases, the presence of the analyte leads to a **decrease** in the fluorophore emission, indicating that there is some interaction between the small molecules but that it does not result in energy transfer. Pairs that fall into this category:

Phenanthrene (**4**) – Rhodamine (**8**)
Fluorene (**5**) – Rhodamine (**8**)

(c) Fluorophore emission ratios less than 1.

Pyrene (**2**) – Rhodamine (**8**)
Pyrene (**2**) – BODIPY (**9**)
Benzo[*a*]pyrene (**3**) – Rhodamine (**8**)
Benzo[*a*]pyrene (**3**) – BODIPY (**9**)
Fluorene (**5**) – BODIPY (**9**)

In these cases, energy transfer from the analyte to the fluorophore occurs, resulting in amplified fluorophore emission from analyte excitation.

DETAILS FOR ENERGY TRANSFER EXPERIMENTS

All energy transfer efficiencies were calculated using Equation 1:

% Efficiency = (I_{DA}/I_D)*100% (1)

where I_{DA} is the integrated emission of the fluorophore from analyte (PAH or PCB) excitation and I_D is the integrated fluorophore emission from direct fluorophore excitation.

All fluorescence emissions were integrated using Origin 8.5, and were integrated vs. wavenumber on the X-axis.

General procedure for energy transfer experiments:

γ-cyclodextrin hydrate (CAS: 91464-90-3) was obtained from Sigma-Aldrich, and dissolved in phosphate buffered saline (PBS) at pH 7.4 at a 10 mM concentration. Serial dilutions were then performed to yield solutions with 1, 2, 3, 4, 5, 6, 7, 8, and 9 mM γ-cyclodextrin in PBS.

All analytes were dissolved at a concentration of 1 mg/mL in tetrahydrofuran (THF): Anthracene, pyrene, benzo[*a*]pyrene, phenanthrene, fluorene, 4,4'-dichlorobiphenyl, PCB29, and PCB77.

Fluorophore solutions were made as follows:

Rhodamine **8**: 0.1 mg/mL in THF

BODIPY **9**: 0.1 mg/mL in THF

Squaraine **10**: 1 mg/mL in THF

Note: Squaraine trials were predominantly performed on a different spectrometer: a Photon Technology International (PTI) instrument, with lamp model number LPS-220B. Slit widths for this fluorimeter were 2 nm excitation slit width and 2 nm emission slit widths. Detection was done at a right angle to the excitation. As a result of the different machine, a 1 mg/mL solution of squaraine was necessary to achieve a visible fluorescent signal.

2.5 mL of the cyclodextrin solution was transferred to a quartz cuvette, and 20 µL of the analyte solution was added via micropipette. The absorbance and fluorescence spectra of the solution were recorded. The fluorophore was then added sequentially in 20 µL increments (up to 100 µL), and the absorbance and fluorescence spectra were recorded after each addition. The final concentrations of each analyte and fluorophore are shown in the tables below:

Final analyte concentrations:

Compound number	Amount added	Final analyte concentration
1	20 µL	44.9 µM
2	20 µL	39.6 µM
3	20 µL	31.7 µM
4	20 µL	44.9 µM
5	20 µL	48.1 µM
6	20 µL	35.9 µM
7	20 µL	31.3 µM

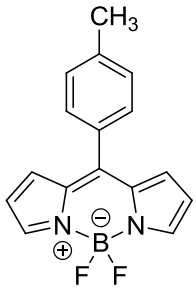
Final fluorophore concentrations:

Compound number	Amount added	Final fluorophore concentration
8	20 µL	1.7 µM
	40 µL	3.3 µM
	60 µL	5.0 µM
	80 µL	6.7 µM
	100 µL	8.4 µM
9	20 µL	1.9 µM
	40 µL	3.8 µM
	60 µL	5.7 µM
	80 µL	7.6 µM
	100 µL	9.5 µM
10	20 µL	10.6 µM
	40 µL	21.2 µM
	60 µL	31.8 µM
	80 µL	42.4 µM
	100 µL	53.0 µM

For each combination, two fluorescence spectra were recorded: the fluorescence from excitation of the analyte (PAH or PCB) and the fluorescence spectra from excitation of the fluorophore. The excitation wavelengths were chosen to be as close as possible to the maximum wavelength of absorption, without significantly truncating the emission spectrum. Excitation wavelengths are recorded below:

Anthracene **1**: 360 nm excitation; emission spectrum recorded from 370 nm – 700 nm
Pyrene **2**: 360 nm excitation; emission spectrum recorded from 370 nm – 700 nm
Benzo[*a*]pyrene **3**: 360 nm excitation; emission spectrum recorded from 370 nm – 700 nm
Phenanthrene **4**: 290 nm excitation; emission spectrum recorded from 300 nm – 550 nm
Fluorene **5**: 270 nm excitation; emission spectrum recorded from 280 nm – 570 nm
4,4'-dichlorobiphenyl **6**: 233 nm excitation; emission spectrum recorded from 243 nm – 600 nm
PCB29 **7**: 233 nm excitation; emission spectrum recorded from 243 nm – 600 nm
Rhodamine **8**: 520 nm excitation; emission spectrum recorded from 530 nm – 800 nm
BODIPY **9**: 460 nm excitation; emission spectrum recorded from 470 nm – 800 nm
Squaraine **10**: 620 nm excitation; emission spectrum recorded from 630 nm – 800 nm

EXPERIMENTS WITH UNFUNCTIONALIZED BODIPY **11**



12

The synthesis of BODIPY **11** was performed according to literature procedures.

Cui, A.; Peng, X.; Fan, J.; Chen, X.; Wu, Y.; Guo, B. "Synthesis, spectral properties and photostability of novel boron-dipyrromethene dyes." *J. Photochem. Photobiol. A Chem.* **2007**, *186*, 85-92.

Control experiments with BODIPY **11** (with 10 mM γ-cyclodextrin and different analytes):

In these experiments, BODIPY **11** was excited at 360 nm in the presence and absence of analyte, and the results are shown below. The black line indicates fluorescence emission from excitation of the BODIPY at 360 nm in the absence of analyte, and the black line indicates fluorescence emission from excitation of the BODIPY/analyte mixture at 360 nm. These results are quantified in Table S1, where the ratio of fluorophore emission is defined as:

Fluorophore emission via low wavelength excitation in the absence of an analyte/ fluorophore emission via low wavelength excitation in the presence of the analyte.

Values close to 1 indicate that the analyte does not affect the fluorophore emission, and that no energy transfer is occurring between the analyte and fluorophore.

Energy transfer percentage is defined as:

$$\% \text{ Efficiency} = (I_{DA}/I_D)*100\% \qquad (1)$$

where I_{DA} is the integrated emission of the fluorophore from PAH excitation and I_D is the integrated fluorophore emission from direct excitation.

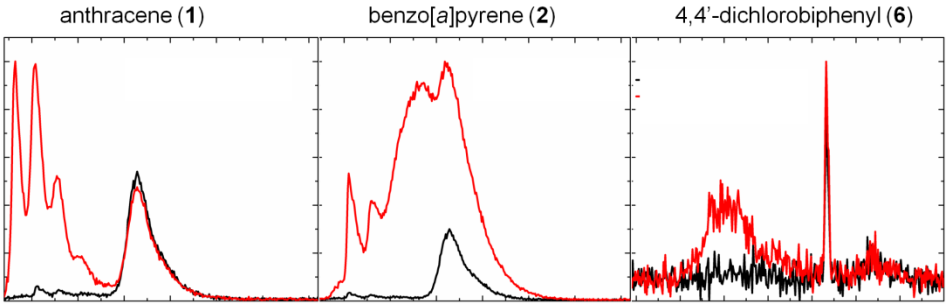
Table S1: Results using BODIPY **11** as a fluorophore in energy transfer schemes.

	Ratio of fluorophore emission	Energy transfer percentage
anthracene (**1**)	1.02	70.7
benzo[*a*]pyrene (**2**)	0.17	397
4,4'-dichlorobiphenyl (**6**)	0.98	40.2

SUMMARY FIGURES FOR BODIPY **11** WITH ANALYTES **1**, **2**, and **6**:

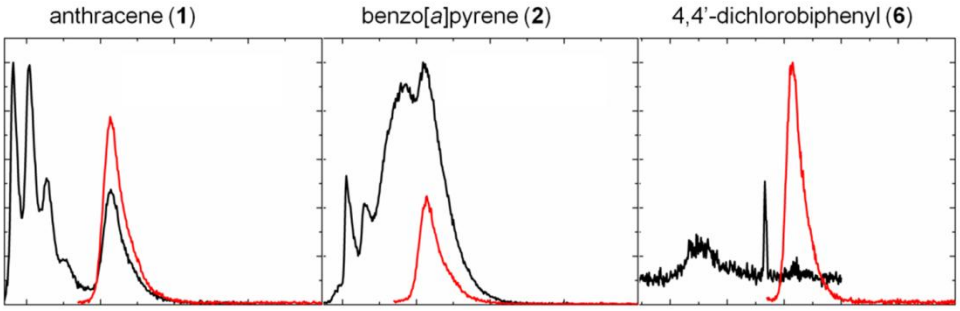
All experiments were done at a 1.5 nm excitation slit width and 1.5 nm emission slit width.

Control experiments exciting at 360 nm in the absence (black) and presence (red) of the analyte.

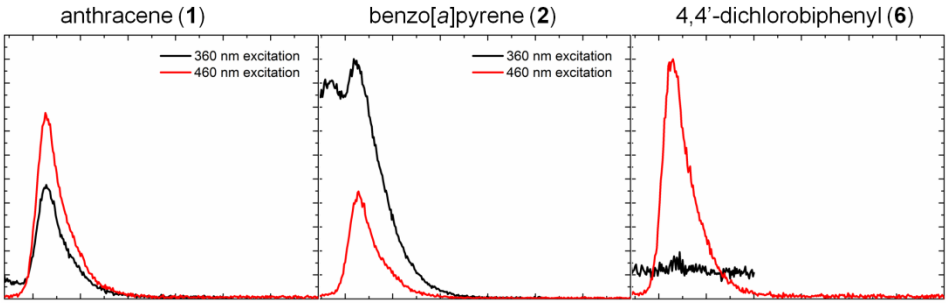


Energy transfer experiments with BODIPY **11** (with 10 mM γ-cyclodextrin and different analytes):

Red line is the excitation of the analyte-BODIPY mixture at 460 nm. Black line is excitation of the analyte-BODIPY mixture at 360 nm.



Zoomed-in figures of energy transfer with BODIPY **11**:



Conclusion: This BODIPY behaves like the thiol-functionalized BODIPY **9**, indicating that the thiol functionality does not interfere with the fluorophore functionality. Like BODIPY **9**, control experiments indicate no significant energy transfer for the anthracene analyte. Significant energy transfer was observed for benzo[*a*]pyrene. No significant energy transfer was observed for 4,4'-dichlorobiphenyl at these slit widths (for higher slit width results, see page 30.)

EXPERIMENTAL DETAILS FROR 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.

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 μL 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. Four repeat measurements were made for the fluorescence emission spectra.

2. 20 μL 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. Four repeat measurements were taken.

3. Step 2 was repeated for 40 μL of analyte, 60 μL of analyte, 80 μL of analyte, and 100 μL 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 mM) 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 mM, which was converted into parts per million (ppm) to better compare with FDA and EPA recommended concentration limits.

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 mM. This LOQ was then converted into parts per million (ppm).

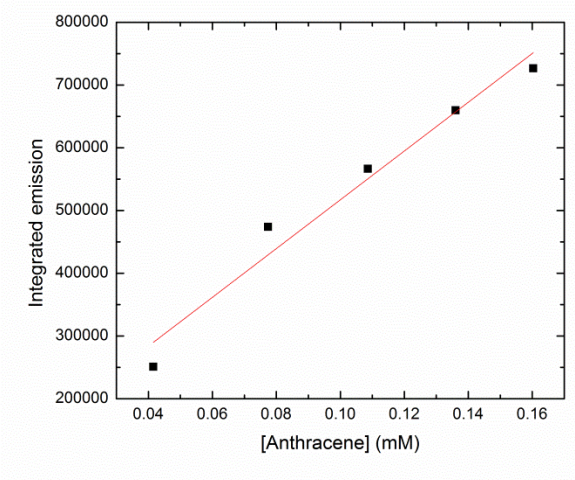
Summary Table for LOD experiments:

Fluorophore	Analyte	Equation	R^2	Limit of Detection (ppm)	Limit of Quantification (ppm)
8	**1**	y = (4E6)X + (1.28E5)	0.968	*a*	8.11
	2	y = (2E7)X -(4.58E5)	0.9497	5.86	7.77
	3	y = (-7E6)X + (3E6)	0.9212	103.77	96.95
	4	y = (-2E6)X + (1E6)	0.6441	83.40	83.30
	5	y = (-2E7)X + (4E6)	0.8448	32.36	32.31
	6	y = (5E6)X -(3.73E4)	0.9076	11.74	12.36
9	**1**	y = (2E6)X + (1.61E5)	0.9498	*a*	*a*
	2	y = (1E7)X + (1.65E5)	0.9687	*a*	*a*
	3	y = (-4E6)X + (1E6)	0.9709	61.42	61.32
	4	y = (-3E6)X + (1E6)	0.8962	55.25	54.51
	5	y = (-2E7)X + (4E6)	0.9059	32.11	31.83
	6	y = (4E6)X + (8.90E5)	0.8142	a	a
	7	y = (5.55E5)X + (6.11E4)	0.9548	9.80	12.90
10	**1**	y = (2E6)X + (4.90E4)	0.9917	*a*	*a*
	2	y = (9E6)X + (3.56E5)	0.9152	*a*	*a*
	3	y = (-8E6)X + (1E6)	0.869	31.09	31.08
	4	y = (-3E6)X + (7.89E5)	0.9093	42.73	42.64

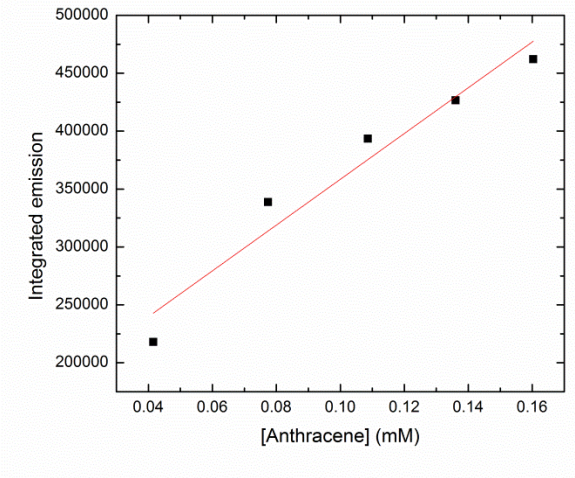
a Attempts to calculate the LOD using these methods resulted in nonsensical values. Current efforts are focused on solving this problem.

Summary Graphs for all LOD experiments:

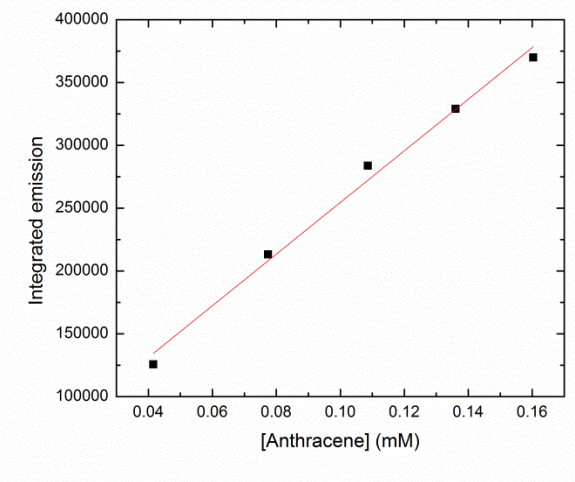
Anthracene (**1**) – Rhodamine (**8**)



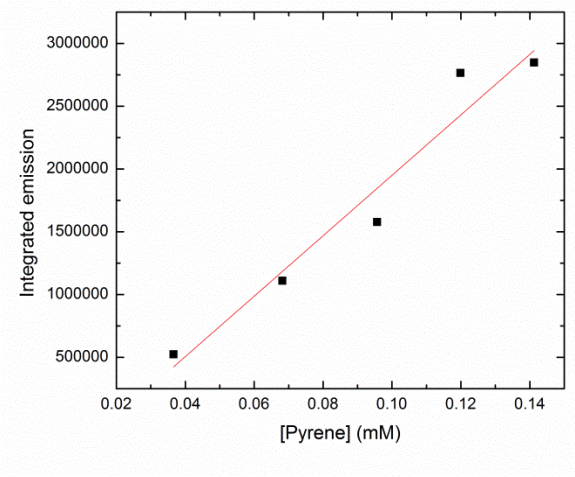
Anthracene (**1**) – BODIPY (**9**)



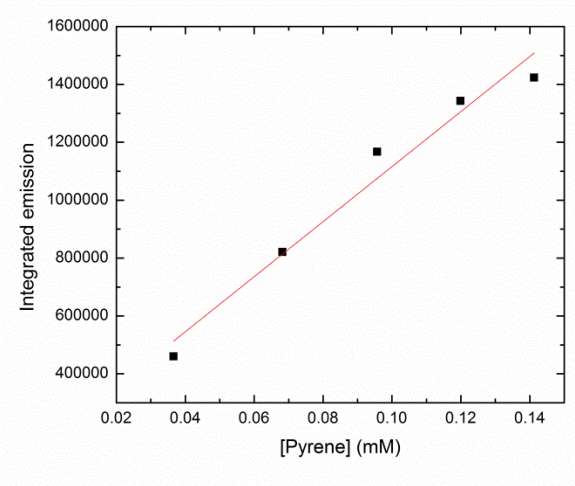
Anthracene (**1**) – Squaraine (**10**)



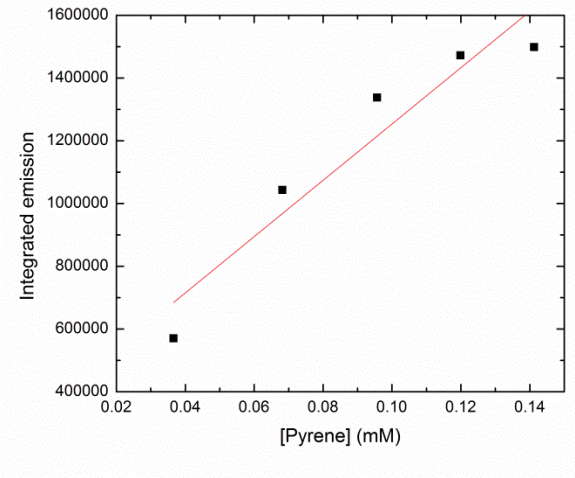
Pyrene (**2**) – Rhodamine (**8**)



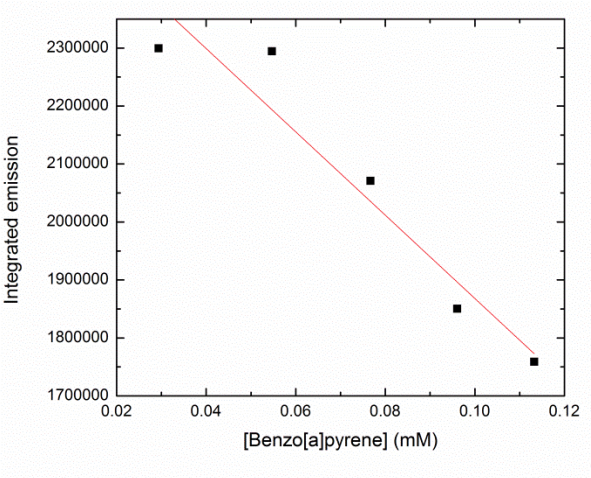
Pyrene (**2**) – BODIPY (**9**)



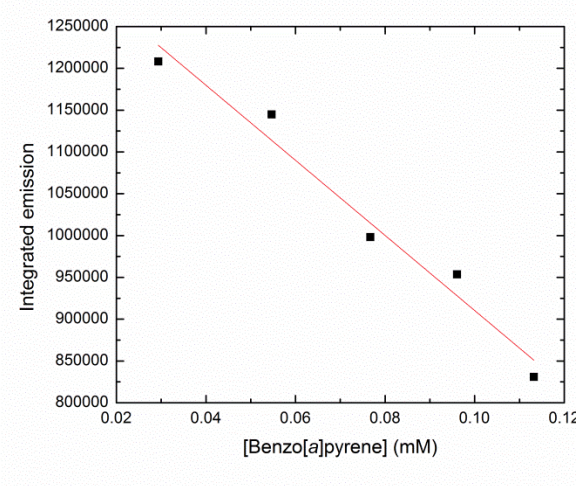
Pyrene (**2**) – Squaraine (**10**)



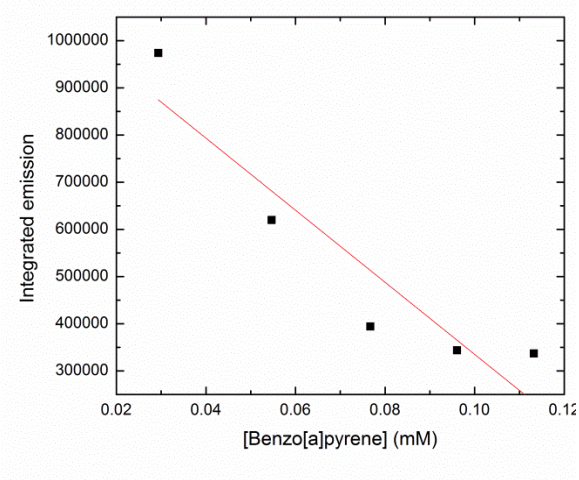
Benzo[*a*]pyrene (**3**) – Rhodamine (**8**)



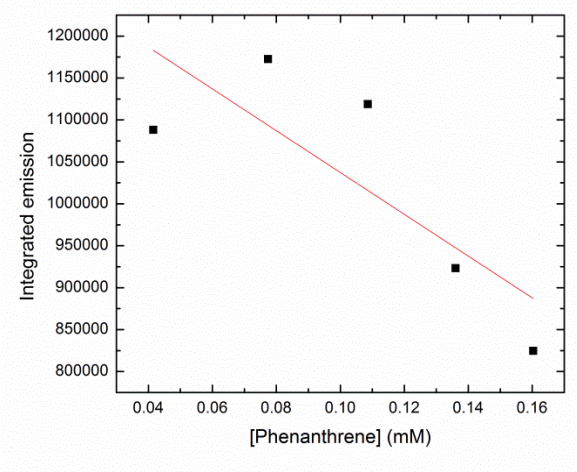
Benzo[*a*]pyrene (**3**) – BODIPY (**9**)



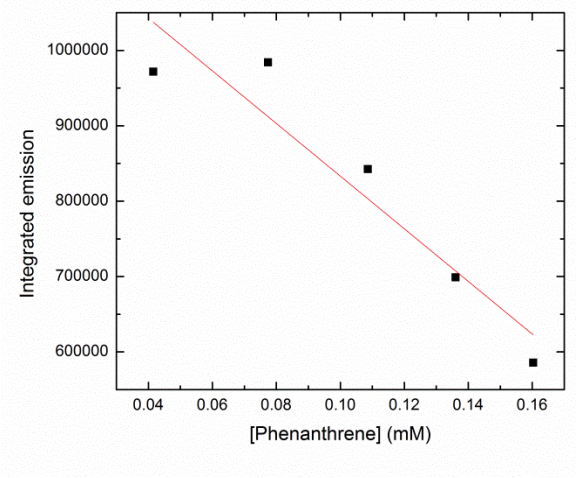
Benzo[*a*]pyrene (**3**) – Squaraine (**10**)



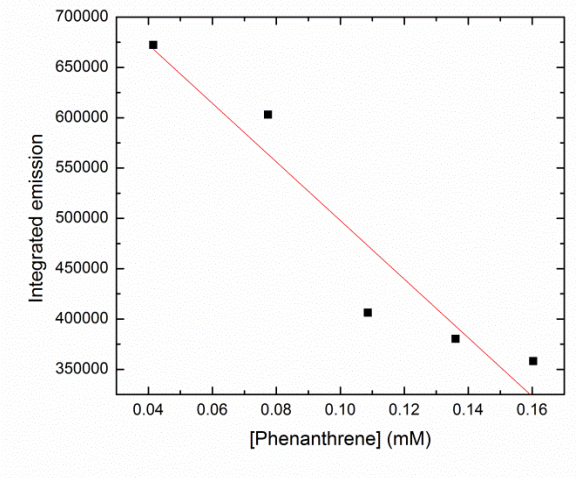
Phenanthrene (**4**) – Rhodamine (**8**)



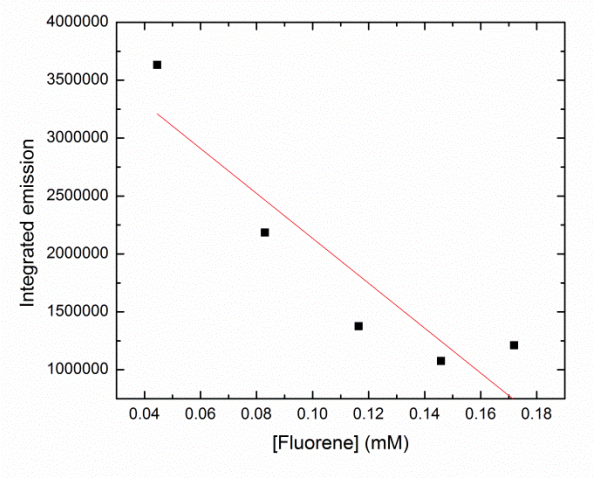
Phenanthrene (**4**) – BODIPY (**9**)



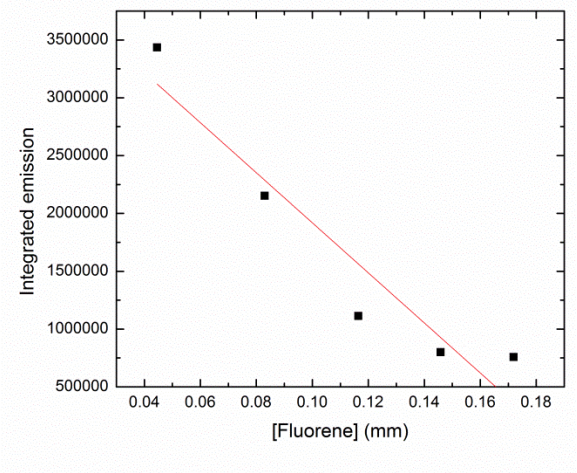
Phenanthrene (**4**) – Squaraine (**10**)



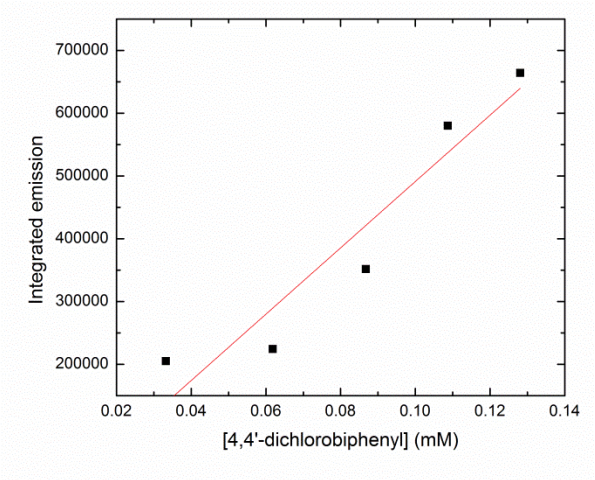
Fluorene (**5**) – Rhodamine (**8**)



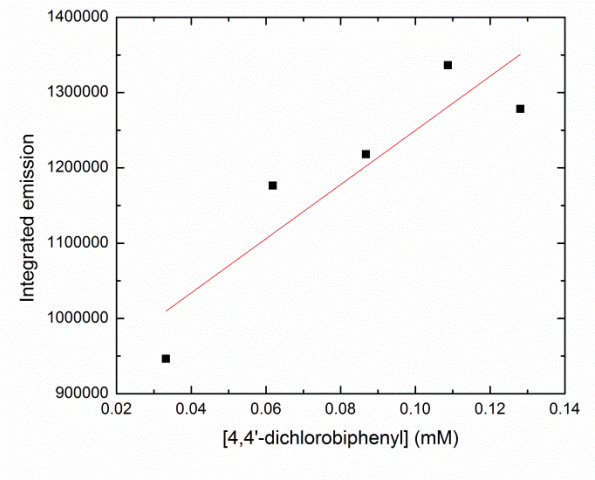
Fluorene (**5**) - BODIPY (**9**)



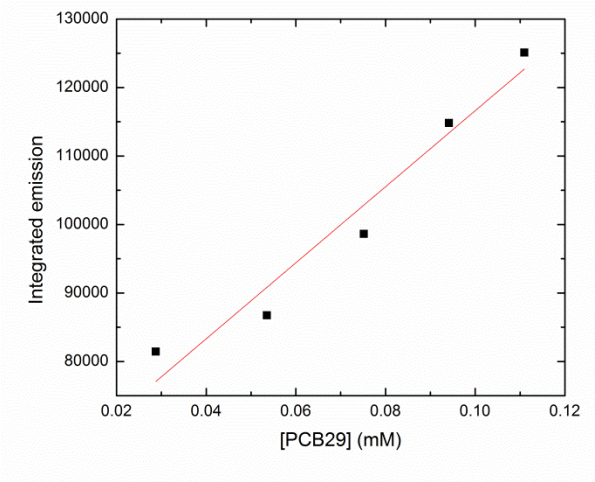
4.4’-dichlorobiphenyl (**6**) - Rhodamine (**8**)



4.4’-dichlorobiphenyl (**6**) - BODIPY (**9**)



PCB29 (**7**) - BODIPY (**9**)



% ENERGY TRANSFER EFFICIENCES FOR ALL ANALYTE-FLUOROPHORE COMBINATIONS:

The highest energy transfer efficiencies are highlighted in bold in each table.

Anthracene (**1**) – Rhodamine (**8**):

	100 µL dye
1 mM γ-CD	8.6
2 mM γ-CD	8.6
3 mM γ-CD	8.7
4 mM γ-CD	8.9
5 mM γ-CD	8.9
6 mM γ-CD	9.3
7 mM γ-CD	9.3
8 mM γ-CD	**9.7**
9 mM γ-CD	9.3
10 mM γ-CD	9.1

Anthracene (**1**) – BODIPY (**9**):

	100 µL dye
1 mM γ-CD	45.5
2 mM γ-CD	57.4
3 mM γ-CD	46.8
4 mM γ-CD	42.5
5 mM γ-CD	46.0
6 mM γ-CD	**71.6**
7 mM γ-CD	59.5
8 mM γ-CD	37.0
9 mM γ-CD	45.4
10 mM γ-CD	34.1

Anthracene (**1**) – Squaraine (**10**):

Refer to Reference 12: T. Mako, P. Marks, N. Cook and M. Levine, *Supramol. Chem.*, 2012, **24**, 743.

Pyrene (**2**) – Rhodamine (**8**):

	100 µL
0 mM γ-CD	3.4
1 mM γ-CD	3.5
2 mM γ-CD	4.9
3 mM γ-CD	5.8
4 mM γ-CD	**6.0**
5 mM γ-CD	4.4
6 mM γ-CD	5.7
7 mM γ-CD	5.7
8 mM γ-CD	5.5
9 mM γ-CD	5.5
10 mM γ-CD	4.6

Pyrene (**2**) – BODIPY (**9**): No tables because the fluorophore emission overlaps significantly with the pyrene excimer emission (see composite figures)

Pyrene (**2**) – Squaraine (**10**):

Refer to Reference 12: T. Mako, P. Marks, N. Cook and M. Levine, *Supramol. Chem.*, 2012, **24**, 743.

Benzo[*a*]pyrene (**3**) – Rhodamine (**8**):

	100 µL
1 mM γ-CD	4.4
2 mM γ-CD	5.4
3 mM γ-CD	7.0
4 mM γ-CD	8.0
5 mM γ-CD	8.0
6 mM γ-CD	8.5
7 mM γ-CD	9.0
8 mM γ-CD	8.8
9 mM γ-CD	9.6
10 mM γ-CD	**10.1**

Benzo[*a*]pyrene (**3**) – BODIPY (**9**): Excessive overlap between the benzo[*a*]pyrene excimer emission and the BODIPY emission.

Benzo[*a*]pyrene (**3**) – Squaraine (**10**):

	100 µL dye
1 mM γ-CD	9.5
2 mM γ-CD	12.1
3 mM γ-CD	16.6
4 mM γ-CD	14.4
5 mM γ-CD	12.3
6 mM γ-CD	12.0
7 mM γ-CD	22.4
8 mM γ-CD	24.1
9 mM γ-CD	**27.4**
10 mM γ-CD	

Phenanthrene (**4**) – Rhodamine (**8**):

	100 µL dye
0 mM γ-CD	4.2
1 mM γ-CD	4.8
2 mM γ-CD	4.0
3 mM γ-CD	4.0
4 mM γ-CD	3.9
5 mM γ-CD	4.2
6 mM γ-CD	3.5
7 mM γ-CD	3.6
8 mM γ-CD	**5.2**
9 mM γ-CD	**5.2**
10 mM γ-CD	4.3

Phenanthrene (**4**) – BODIPY (**9**):

	100 µL dye
1 mM γ-CD	16.7
2 mM γ-CD	**17.2**
3 mM γ-CD	13.8
4 mM γ-CD	12.4
5 mM γ-CD	10.1
6 mM γ-CD	9.1
7 mM γ-CD	10.6
8 mM γ-CD	8.5
9 mM γ-CD	3.5
10 mM γ-CD	10.2

Phenanthrene (**4**) –Squaraine (**10**): Preliminary experiments (1, 5, and 10 mM γ-cyclodextrin) indicate no energy transfer.

Fluorene (**5**) – Rhodamine (**8**):

	100 µL dye
1 mM γ-CD	**3.7**
2 mM γ-CD	3.0
3 mM γ-CD	3.5
4 mM γ-CD	2.9
5 mM γ-CD	2.9
6 mM γ-CD	3.3
7 mM γ-CD	2.8
8 mM γ-CD	3.5
9 mM γ-CD	
10 mM γ-CD	3.2

Fluorene (**5**) – BODIPY (**9**):

	100 µL dye
1 mM γ-CD	5.2
2 mM γ-CD	6.1
3 mM γ-CD	7.6
4 mM γ-CD	7.9
5 mM γ-CD	9.0
6 mM γ-CD	10.5
7 mM γ-CD	9.4
8 mM γ-CD	10.1
9 mM γ-CD	9.3
10 mM γ-CD	**16.4**

Fluorene (**5**) – Squaraine (**10**): Preliminary experiments (1, 5, and 10 mM γ-cyclodextrin) indicate no energy transfer

4,4’-Dichlorobiphenyl (**6**) – Rhodamine (**8**):

	100 µL dye
1 mM γ-CD	**7.8**
2 mM γ-CD	7.6
3 mM γ-CD	6.4
4 mM γ-CD	7.2
5 mM γ-CD	7.9
6 mM γ-CD	7.0
7 mM γ-CD	7.2
8 mM γ-CD	7.4
9 mM γ-CD	7.0
10 mM γ-CD	6.2

4,4’-Dichlorobiphenyl (**6**) – BODIPY (**9**):

	100 µL dye
1 mM γ-CD	7.8
2 mM γ-CD	8.8
3 mM γ-CD	8.1
4 mM γ-CD	8.3
5 mM γ-CD	8.6
6 mM γ-CD	8.5
7 mM γ-CD	9
8 mM γ-CD	8.4
9 mM γ-CD	**9.2**
10 mM γ-CD	**9.2**

4,4'-Dichlorobiphenyl (**6**) – Squaraine (**10**): Preliminary results indicate no energy transfer is observed.

PCB 29 (**7**) – Rhodamine (**8**): Preliminary results indicate that no energy transfer is observed.

PCB 29 (**7**) – BODIPY (**9**):

	100 µL dye
1 mM γ-CD	7.3
2 mM γ-CD	6.9
3 mM γ-CD	7.0
4 mM γ-CD	6.6
5 mM γ-CD	7.4
6 mM γ-CD	7.5
7 mM γ-CD	7.7
8 mM γ-CD	7.4
9 mM γ-CD	8.0
10 mM γ-CD	**8.6**

PCB 29 (**7**) – Squaraine (**10**): Preliminary results indicate no energy transfer is observed.

SUMMARY DATA FOR HIGHER SLIT WIDTHS

For a few cases where the control experiments showed fluorophore emission ratios near 1, we conducted additional control experiments with 3 nm excitation slit width and 3 nm emission slit widths, to ensure that the fluorophore emission was accurately detected. The fluorophore emission ratios are shown in the table below:

10 mM γ-cyclodextrin was used in each case.

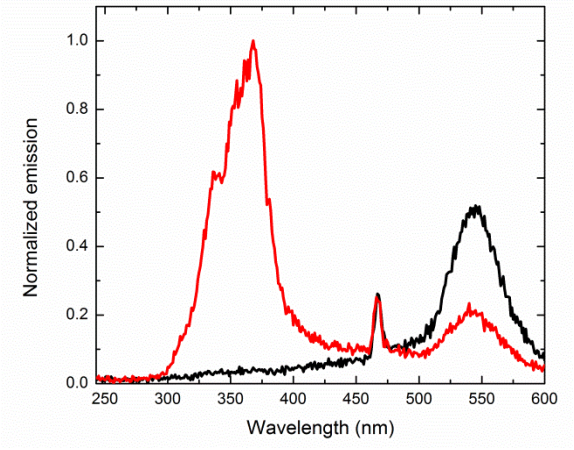
Fluorophore emission ratios:

fluorophore	analyte	Ratio of fluorophore emission
8	**6**	1.99
8	**7**	1.89
9	**6**	1.63
9	**7**	1.78
10	**1**	1.09
10	**2**	0.73
10	**3**	0.91
11	**6**	1.35
11	**7**	1.25

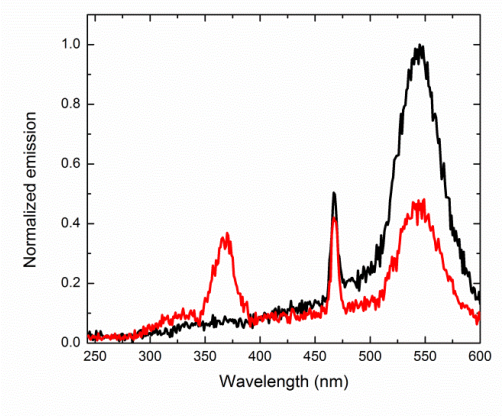
The fluorophore emission ratios for **6-8, 7-8, 6-9** and **7-9** are significantly higher than 1 under these higher slit width conditions, which indicates some interaction (but still no energy transfer, which would be indicated by a value less than 1).

GRAPHS OF CONTROL EXPERIMENTS AT HIGHER SLIT WIDTHS

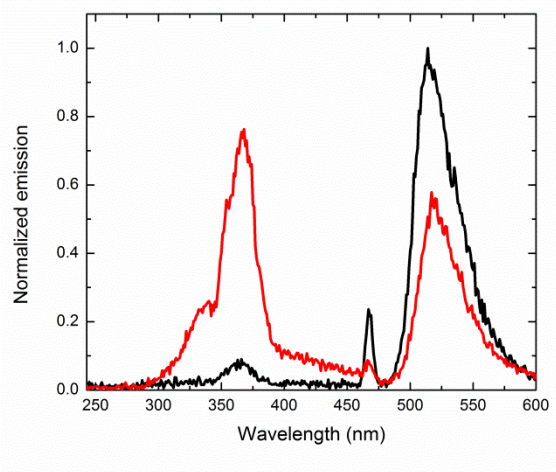
4,4'-dichlorobiphenyl (**6**) – Rhodamine (**8**)



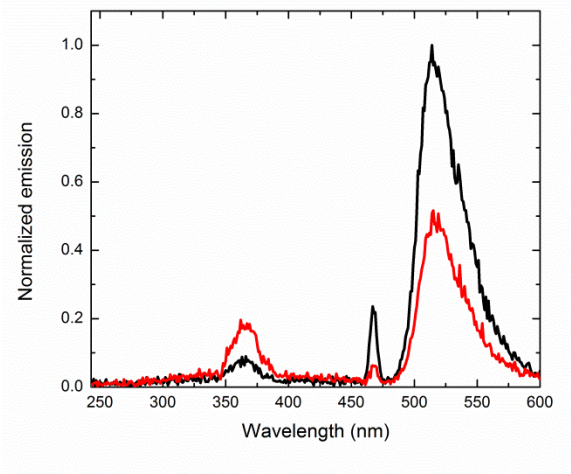
PCB29 (**7**) – Rhodamine (**8**)



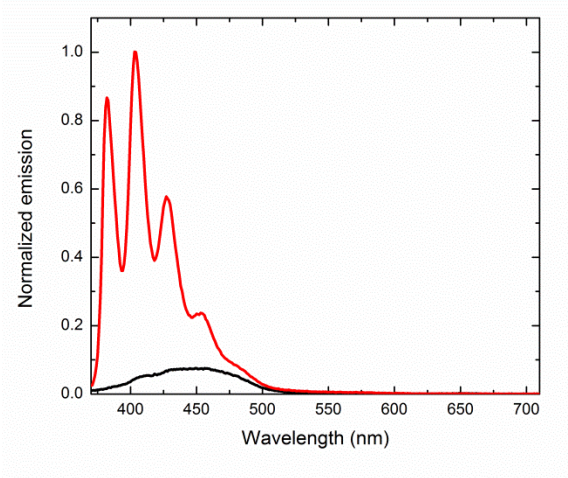
4,4'-dichlorobiphenyl (**6**) – BODIPY (**9**)



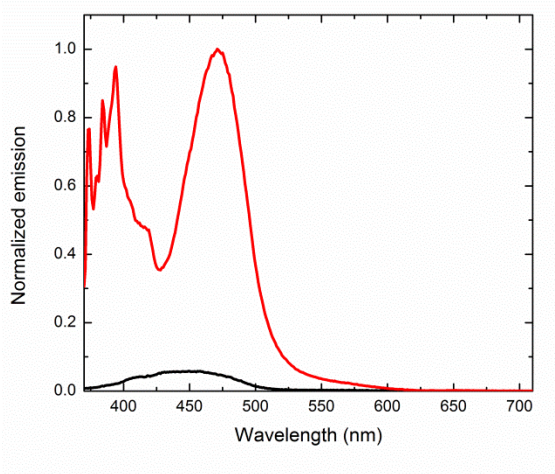
PCB29 (**7**) – BODIPY (**9**)



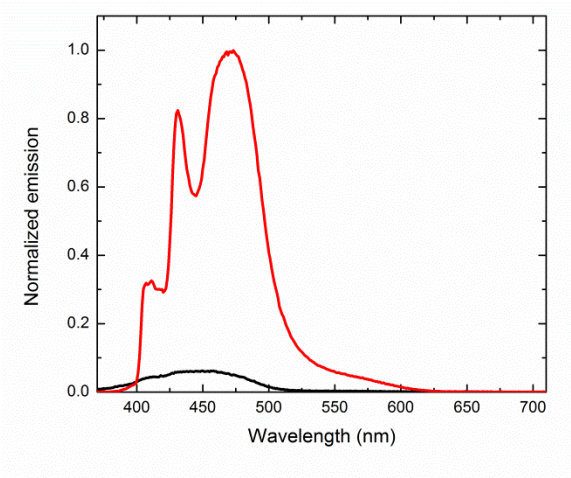
Anthracene (**1**) – Squaraine (**10**)



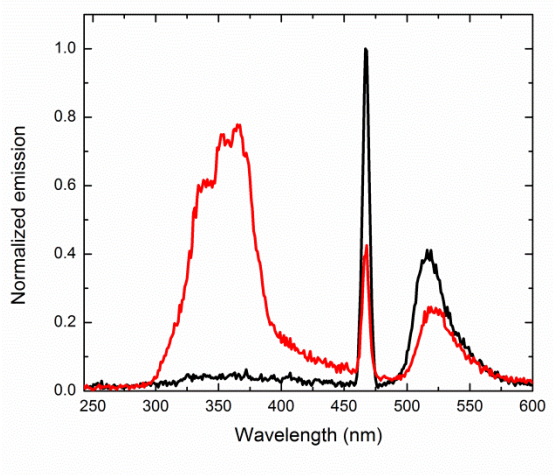
Pyrene (**2**) – Squaraine (**10**)



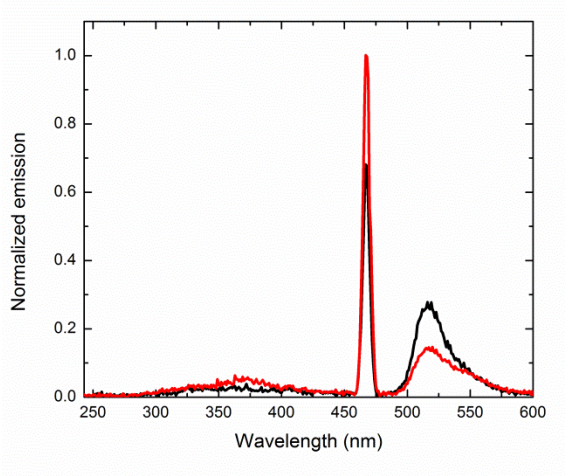
Benzo[*a*]pyrene (**3**) – Squaraine (**10**)



4,4'-dichlorobiphenyl (**6**) – BODIPY (**11**)



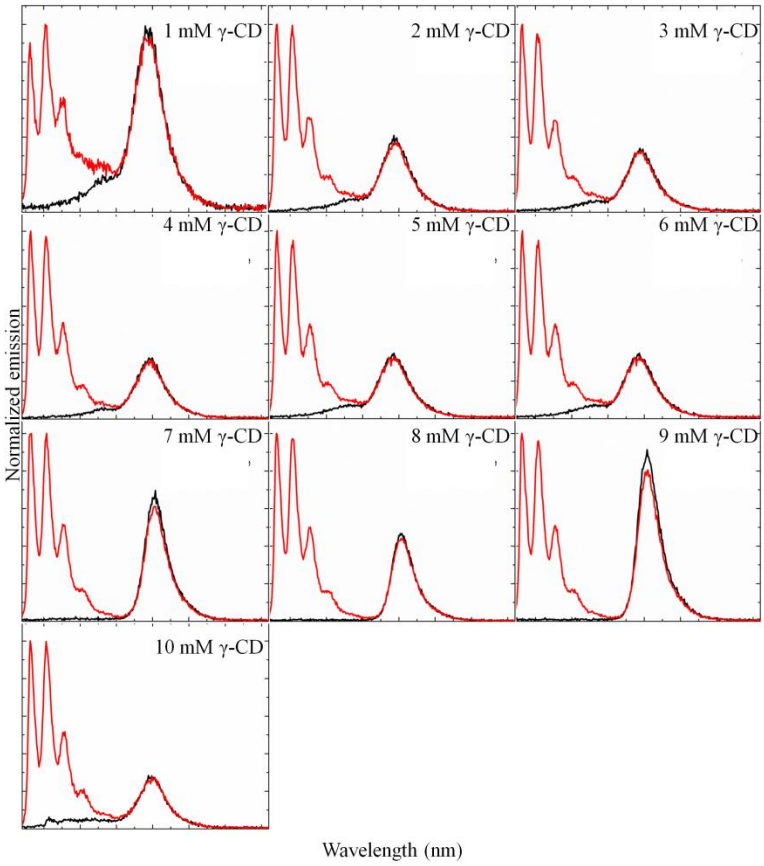
PCB29 (**7**) – BODIPY (**11**)



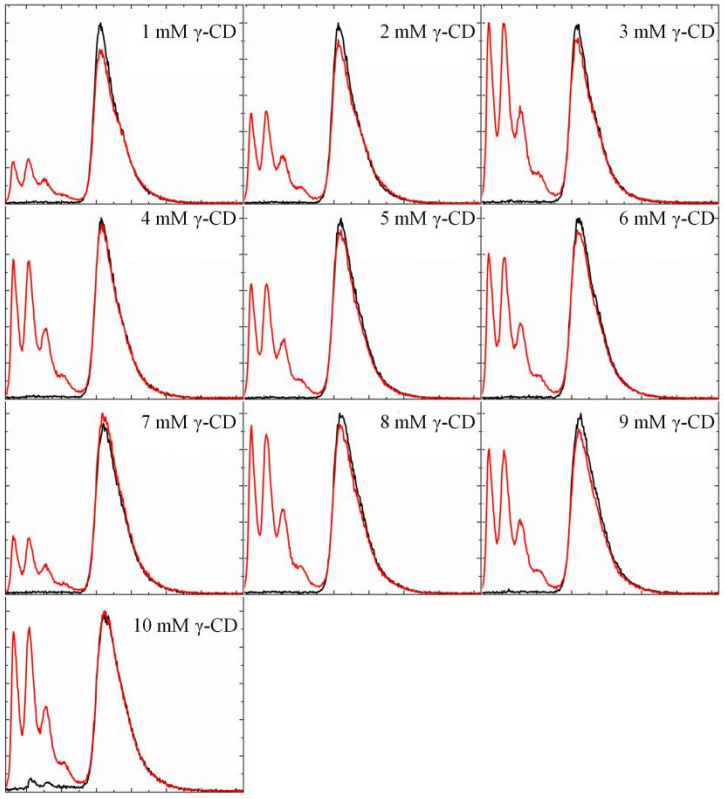
SUMMARY FIGURES FOR ALL CONTROL EXPERIMENTS

Summary graphs for all control experiments. All cases show the fluorophore emission in the absence of analyte (black) and in the presence of analyte (red), with excitation wavelengths as indicated for each summary graph.

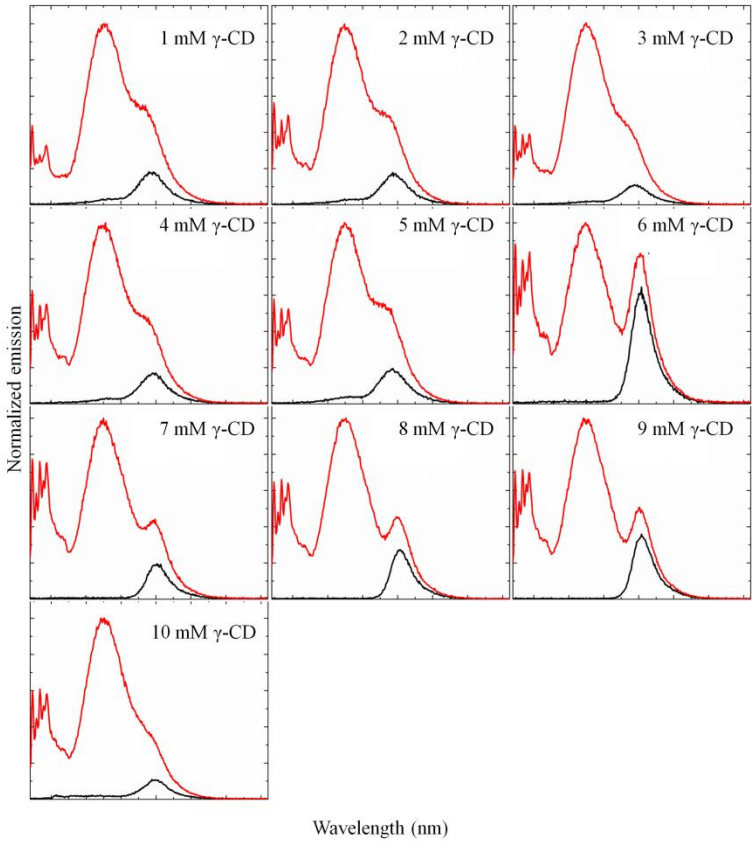
Anthracene (**1**) – Rhodamine (**8**) (360 nm excitation)



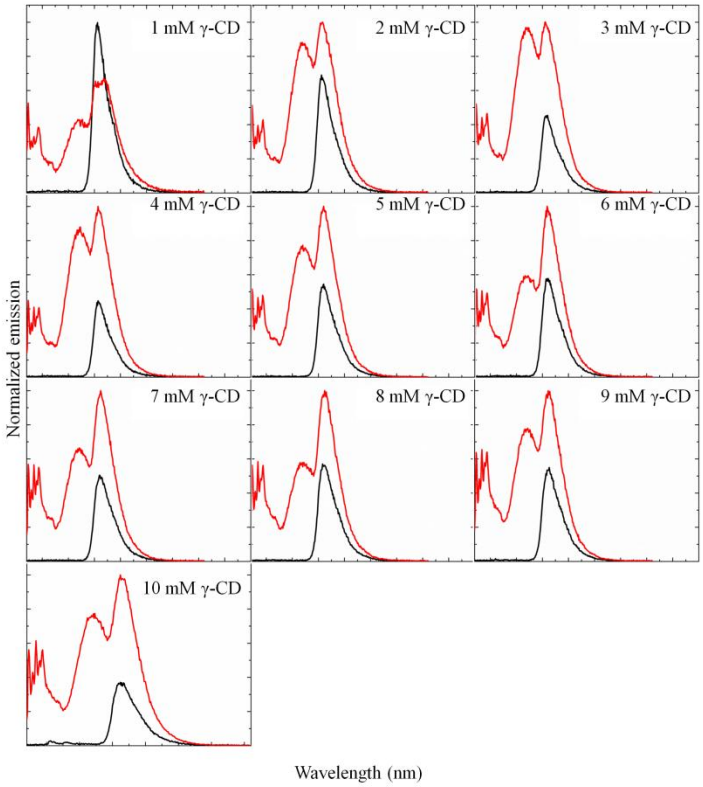
Anthracene (**1**) – BODIPY (**9**) (360 nm excitation)



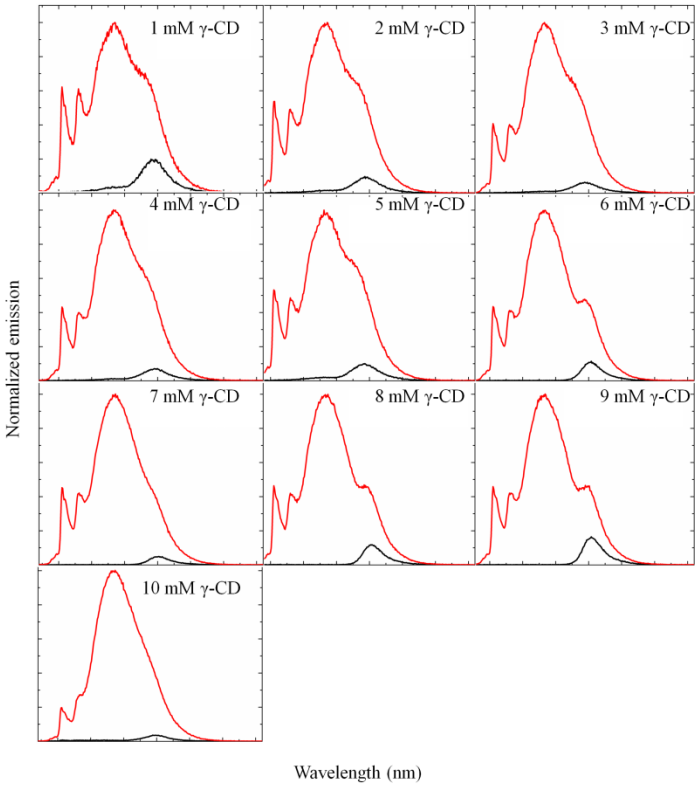
Pyrene (**2**) – Rhodamine (**8**) (360 nm excitation)



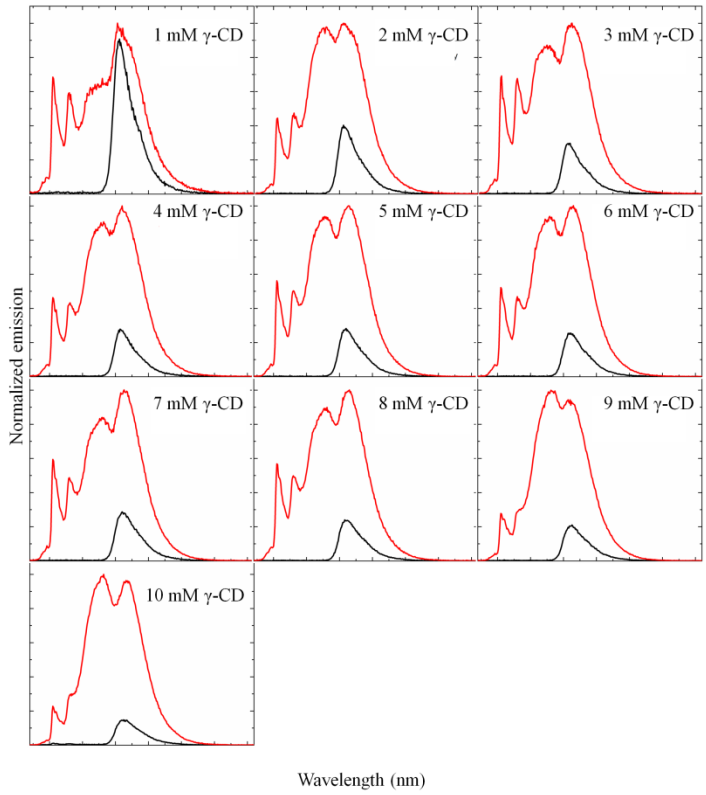
Pyrene (**2**) – BODIPY (**9**) (360 nm excitation)



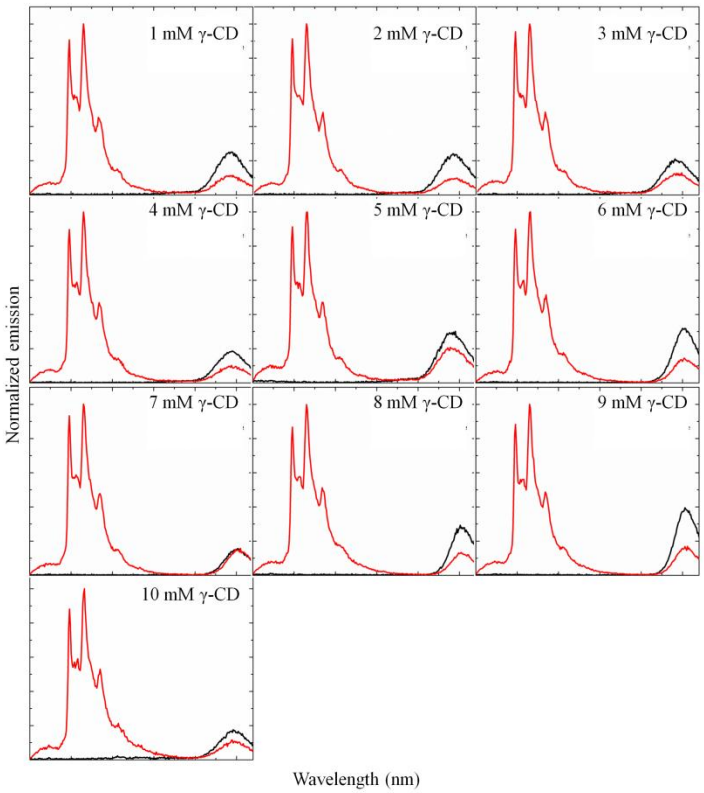
Benzo[*a*]pyrene (**3**) – Rhodamine (**8**) (360 nm excitation)



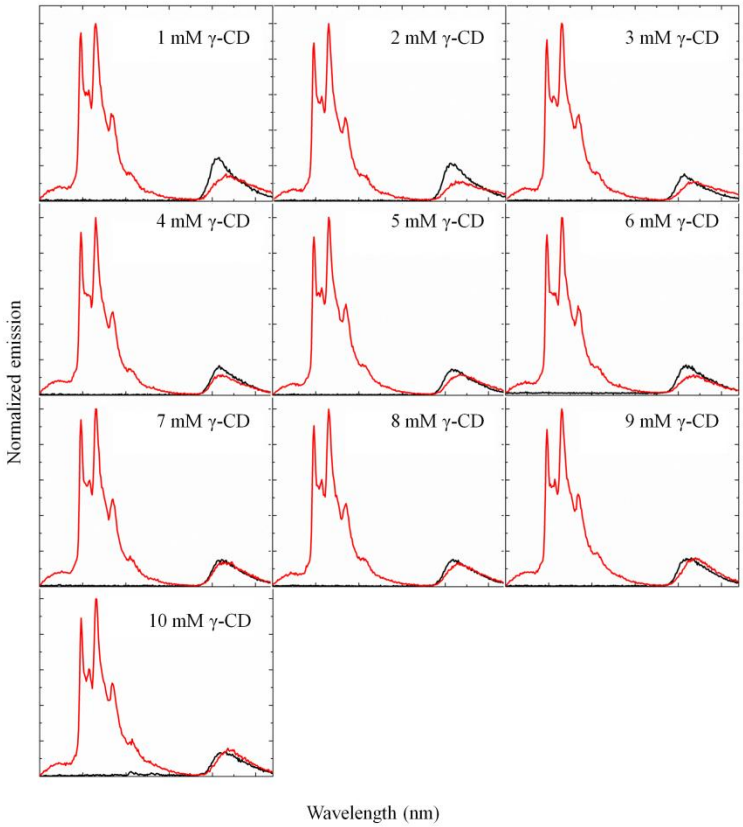
Benzo[*a*]pyrene (**3**) – BODIPY (**9**) (360 nm excitation)



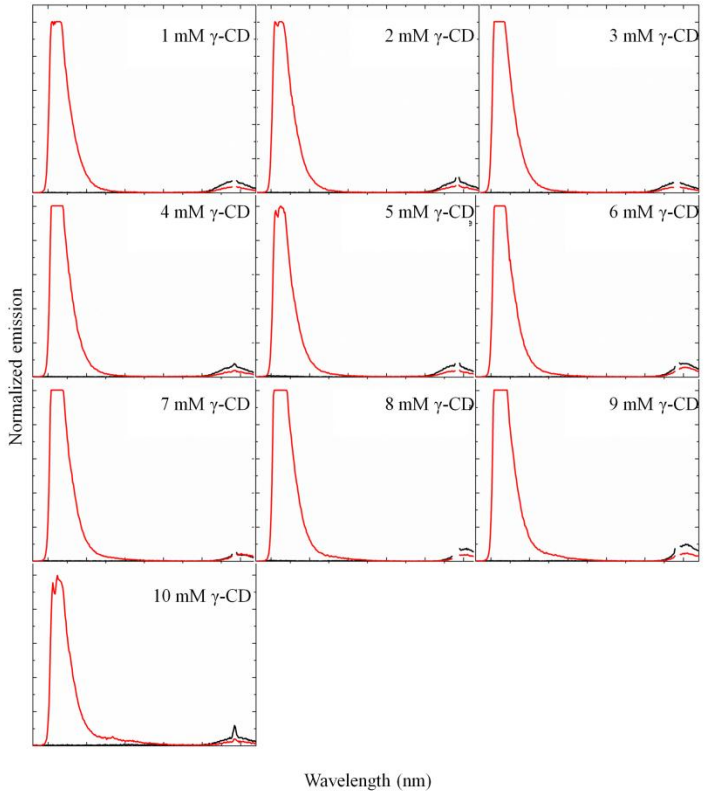
Phenanthrene (**4**) – Rhodamine (**8**) (360 nm excitation)



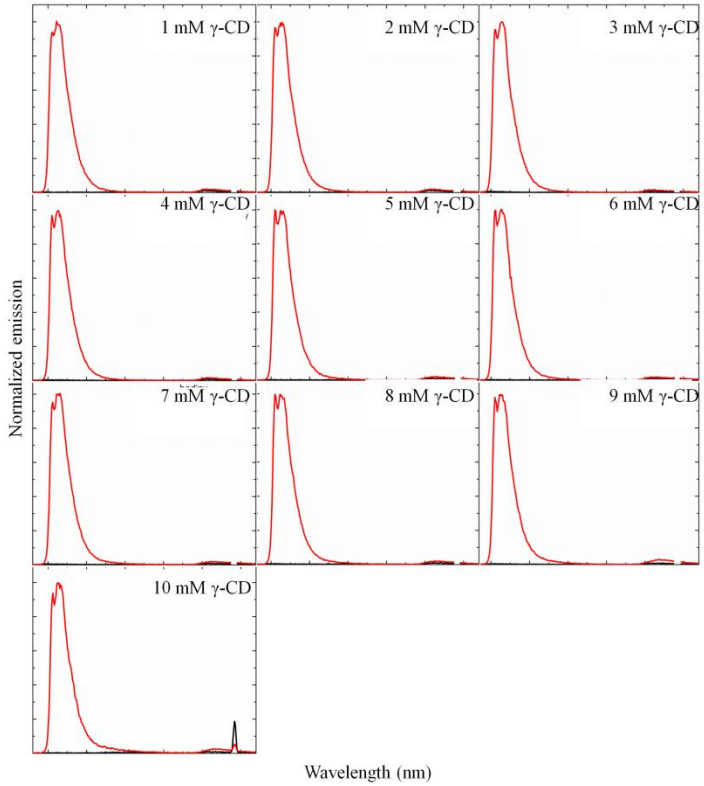
Phenanthrene (**4**) – BODIPY (**9**)



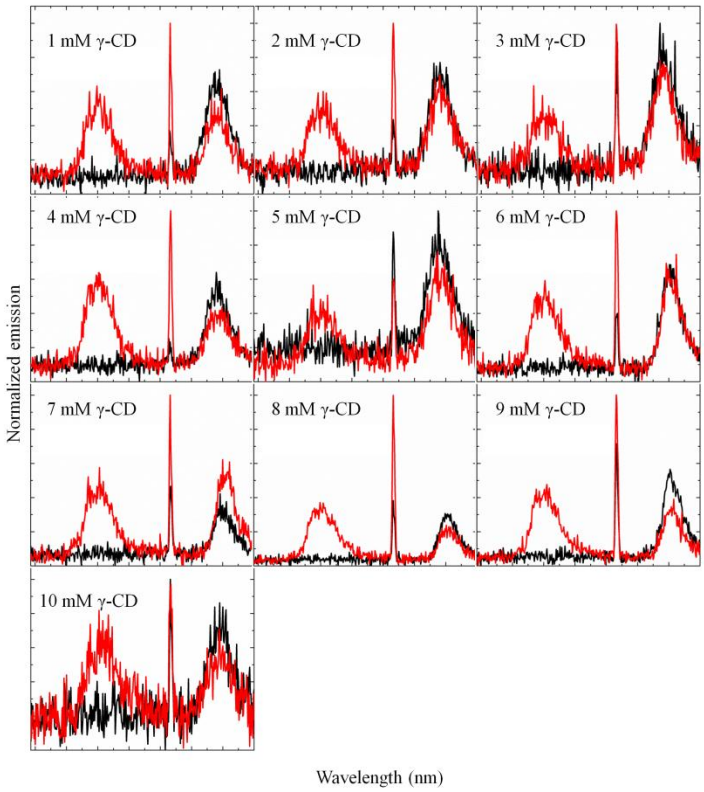
Fluorene (**5**) – Rhodamine (**8**)



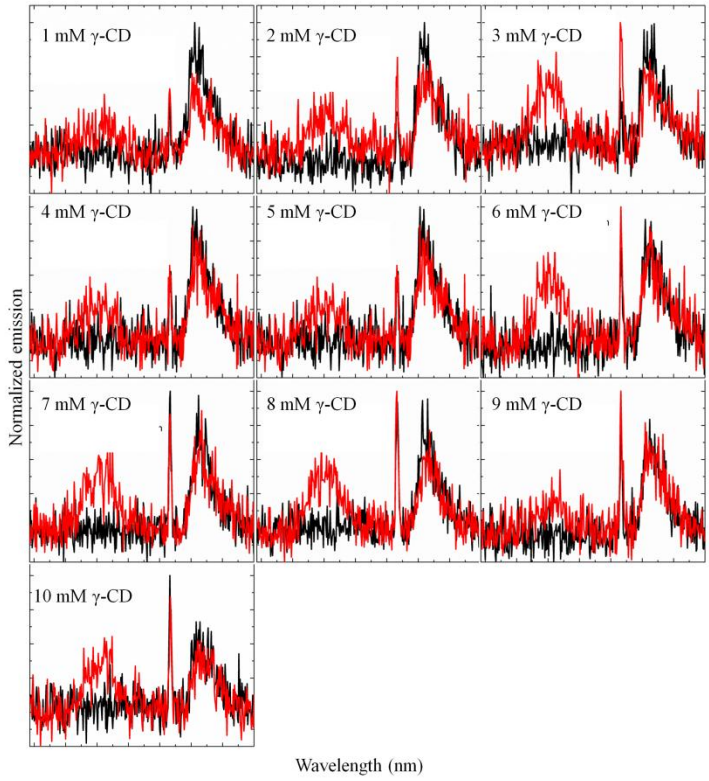
Fluorene (**5**) – BODIPY (**9**)



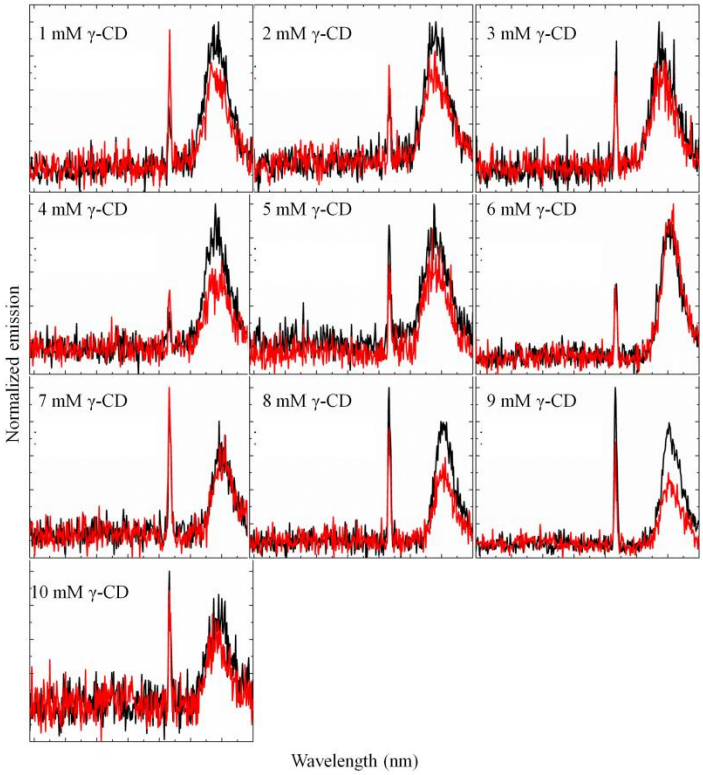
4,4’-dichlorobiphenyl (**6**) – Rhodamine (**8**)



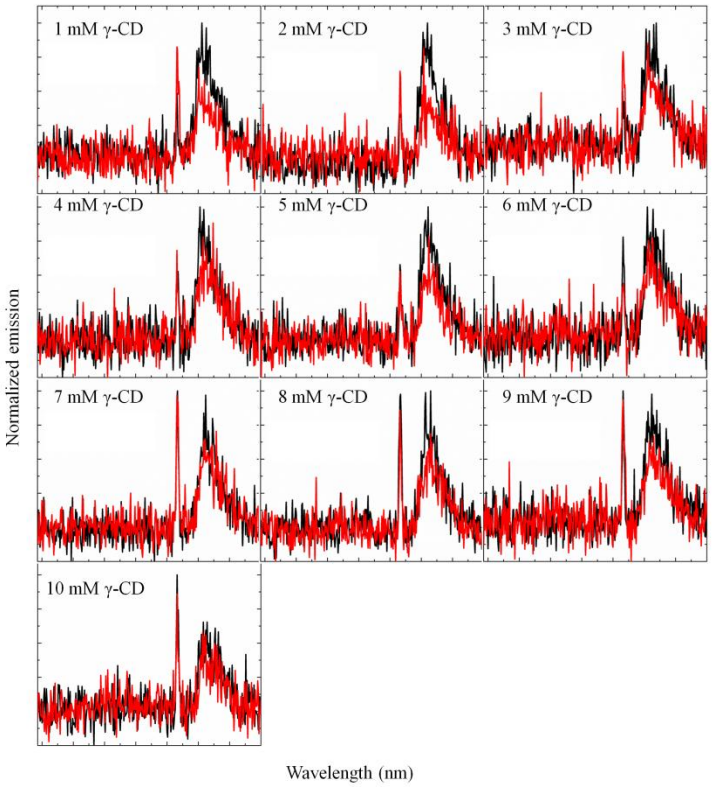
4,4'-dichlorobiphenyl (**6**) – BODIPY (**9**)



PCB29 (**7**) – Rhodamine (**8**)



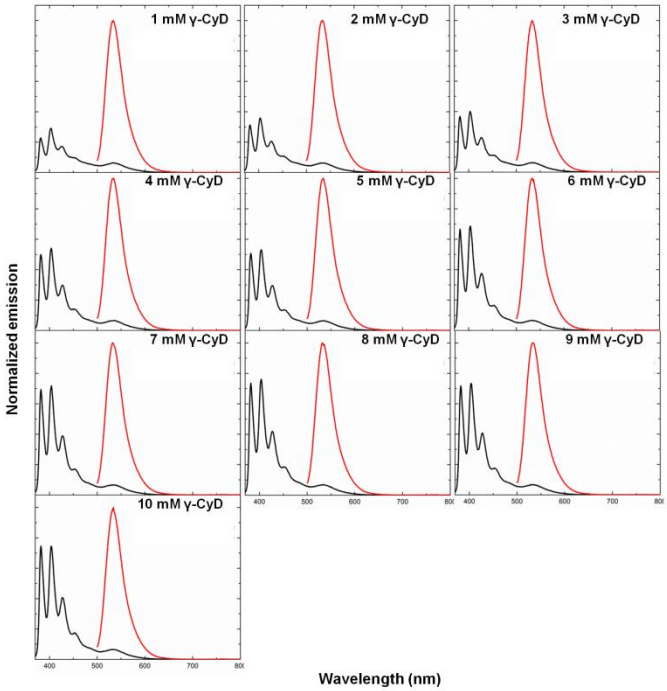
PCB29 (**7**) – BODIPY (**9**)



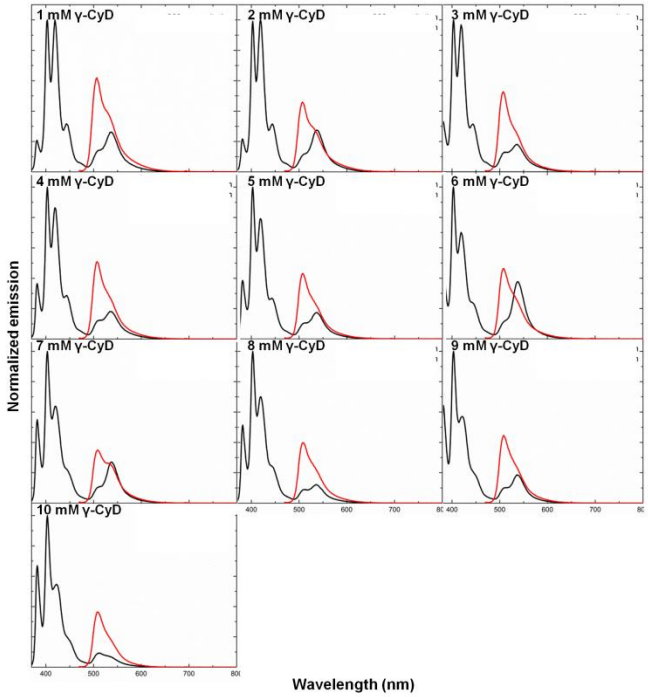
SUMMARY FIGURES FOR ALL ANALYTE-FLUOROPHORE COMBINATIONS:

Figures are at the maximum fluorophore concentration unless otherwise noted. The black line represents the fluorescence from excitation of the analyte, and the red line represents the fluorescence from direct fluorophore excitation.

Anthracene (**1**) – Rhodamine (**8**):



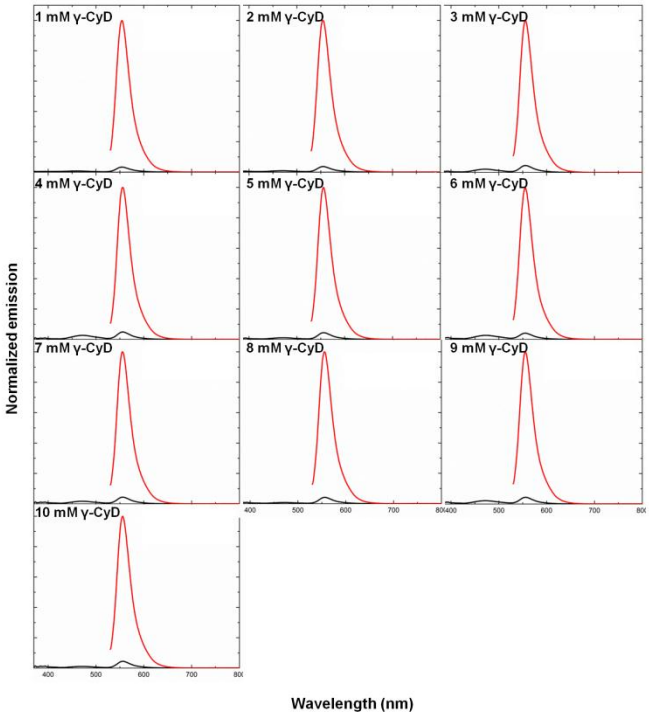
Anthracene (**1**) – BODIPY (**9**):



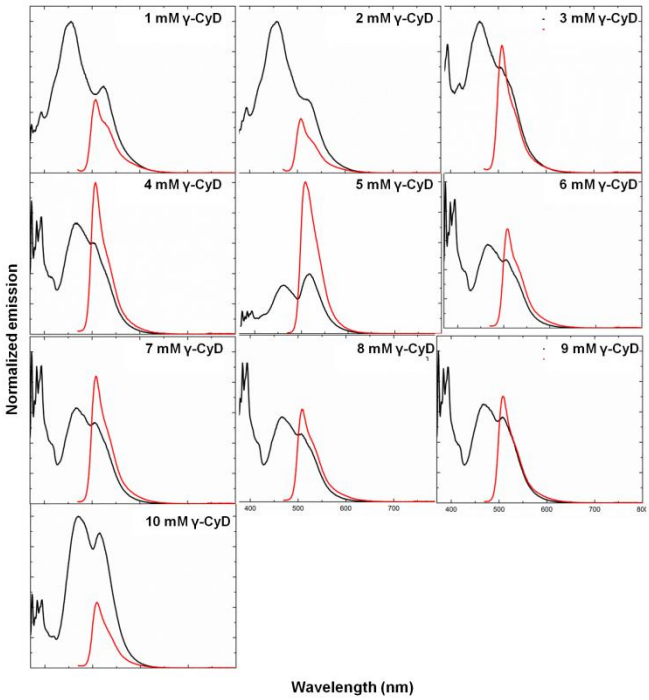
Anthracene (**1**) – Squaraine (**10**):

Refer to Reference 12: T. Mako, P. Marks, N. Cook and M. Levine, *Supramol. Chem.*, 2012, **24**, 743.

Pyrene (**2**) – Rhodamine (**8**):



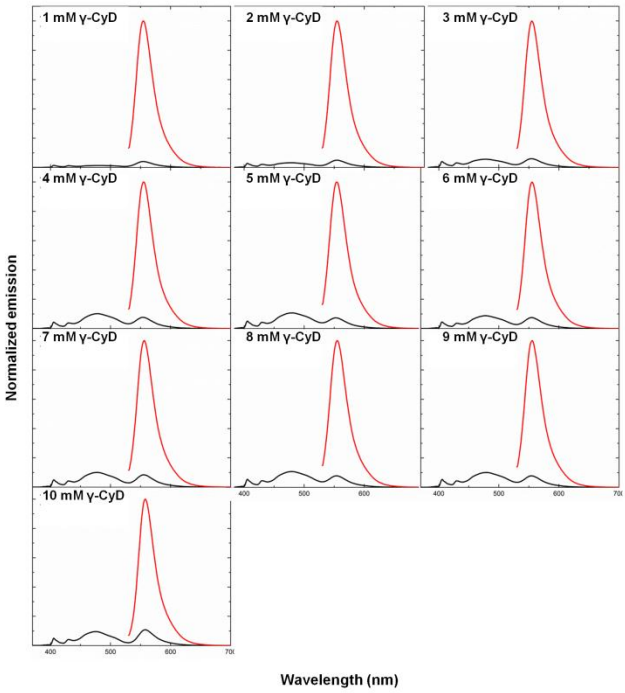
Pyrene (**2**) – BODIPY (**9**):



Pyrene (**2**) – Squaraine (**10**):

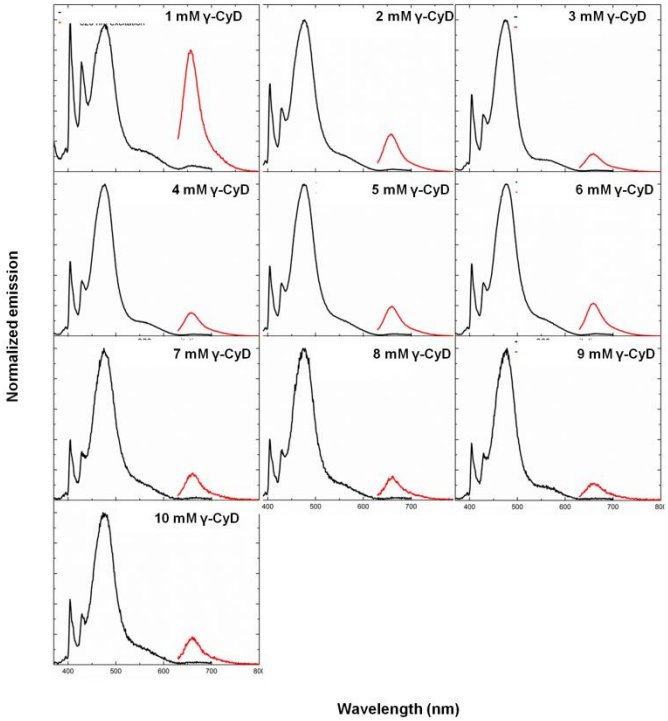
Refer to Reference 12: T. Mako, P. Marks, N. Cook and M. Levine, *Supramol. Chem.*, 2012, **24**, 743.

Benzo[*a*]pyrene (**3**) – Rhodamine (**8**):

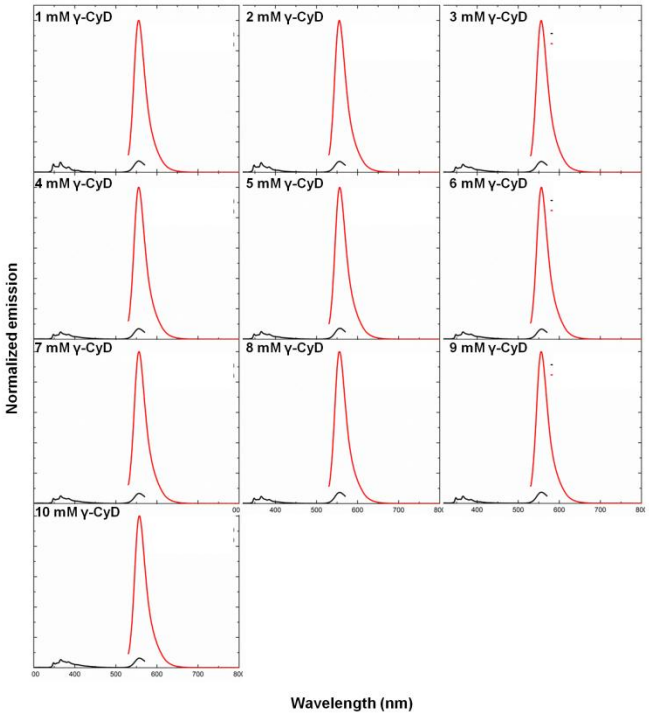


Benzo[*a*]pyrene (**3**) – BODIPY (**9**): Excessive overlap between the benzo[*a*]pyrene excimer emission and the BODIPY emission.

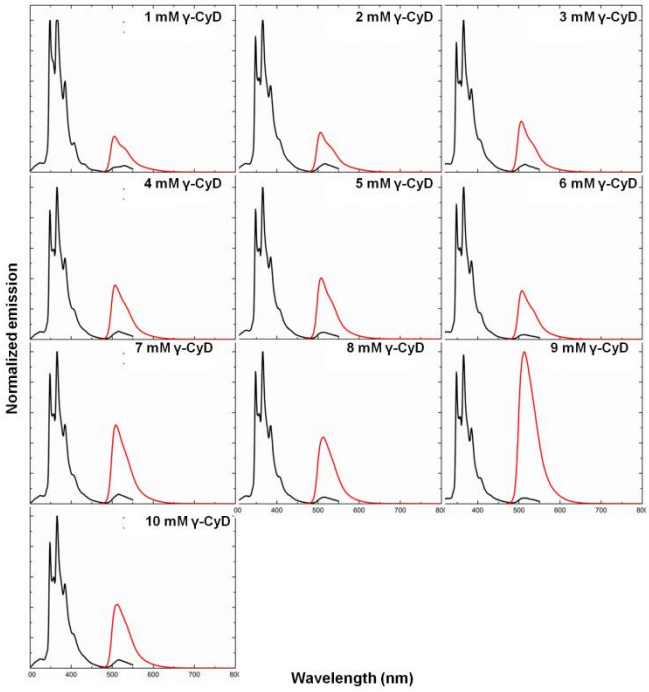
Benzo[*a*]pyrene (**3**) – Squaraine (**10**):



Phenanthrene (**4**) – Rhodamine (**8**):

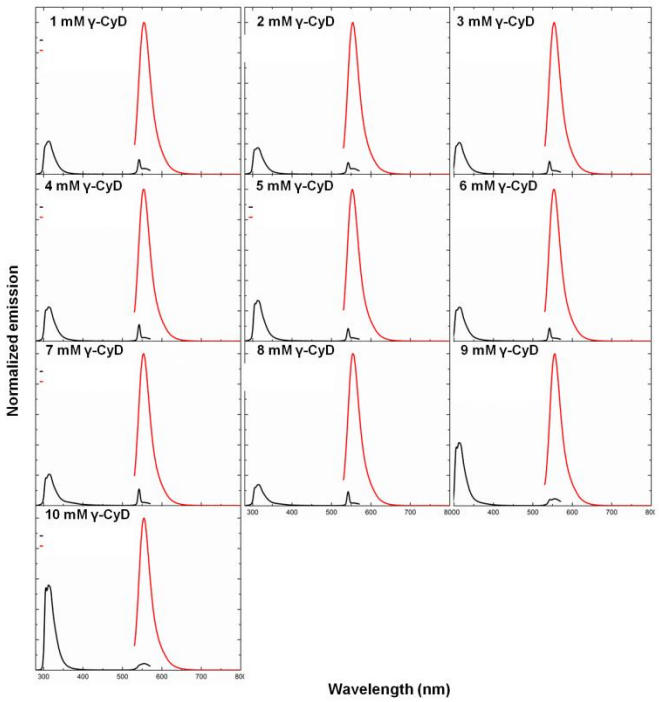


Phenanthrene (**4**) – BODIPY (**9**):

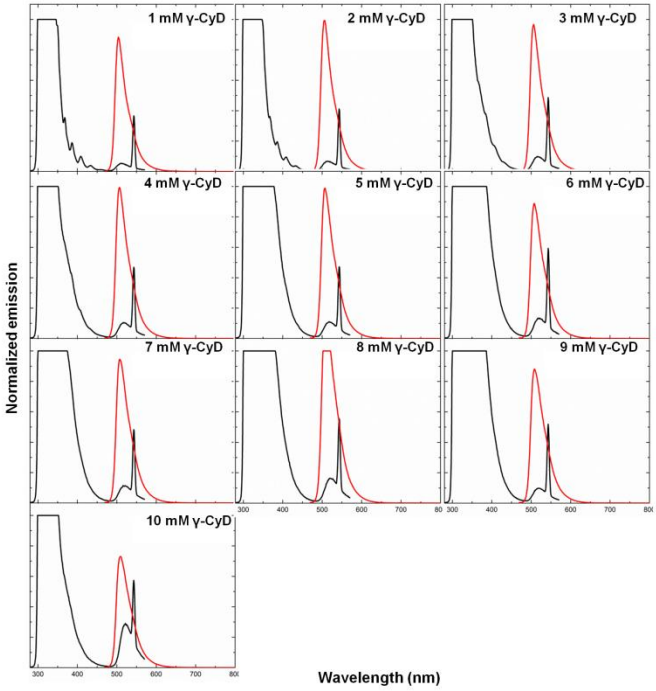


Phenanthrene (**4**) – Squaraine (**10**): Preliminary results indicate no energy transfer.

Fluorene (**5**) – Rhodamine (**8**):

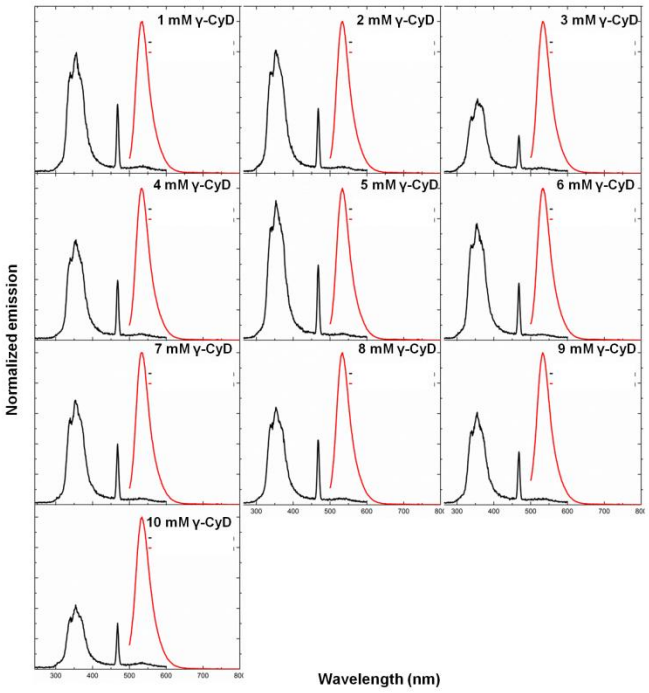


Fluorene (**5**) – BODIPY (**9**):

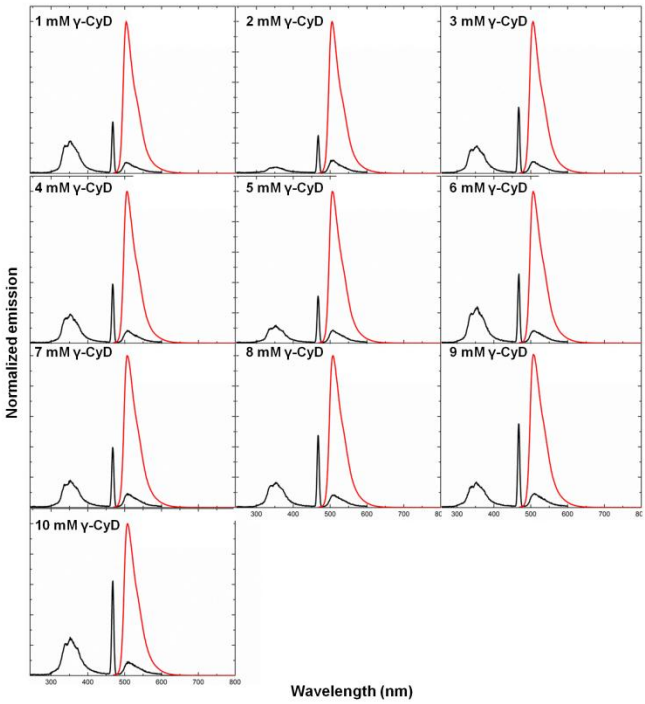


Fluorene (**5**) – Squaraine (**10**): Preliminary results indicate no energy transfer is observed.

4,4'-Dichlorobiphenyl (**6**) – Rhodamine (**8**):



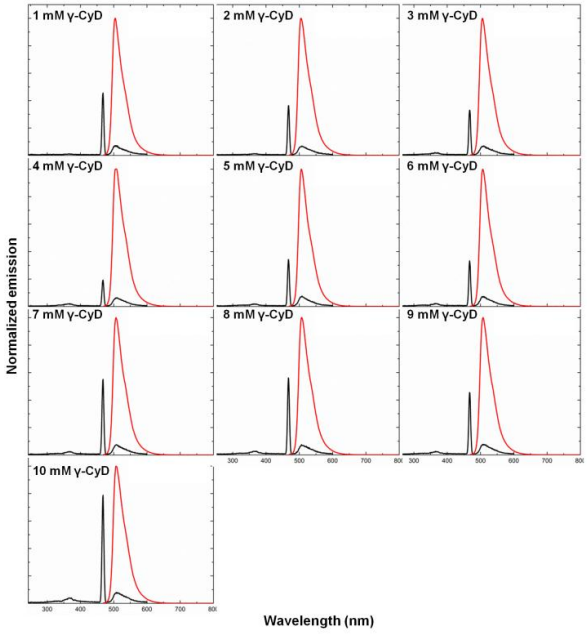
4,4'-Dichlorobiphenyl (**6**) – BODIPY (**9**):



4,4'-Dichlorobiphenyl (**6**) – Squaraine (**10**): Preliminary results indicate no energy transfer is observed.

PCB 29 (**7**) – Rhodamine (**8**): Preliminary results indicate no energy transfer is observed.

PCB 29 (**7**) – BODIPY (**9**):

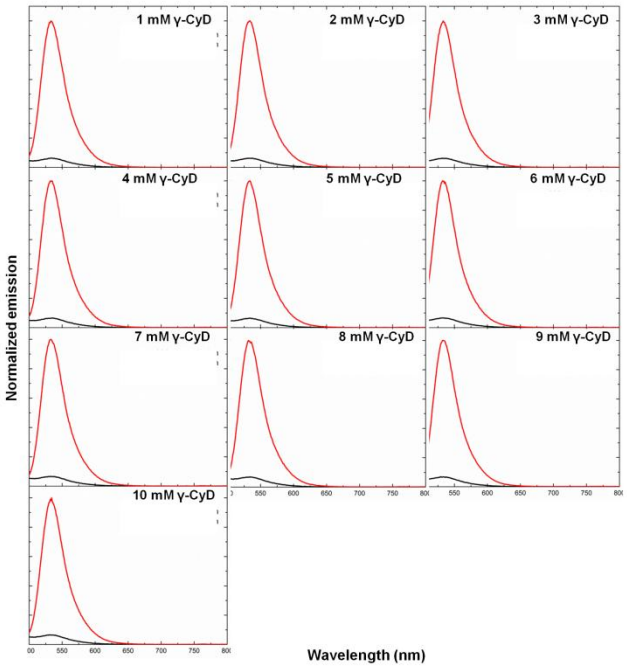


PCB 29 (**7**) – Squaraine (**10**): Preliminary results indicate no energy transfer is observed.

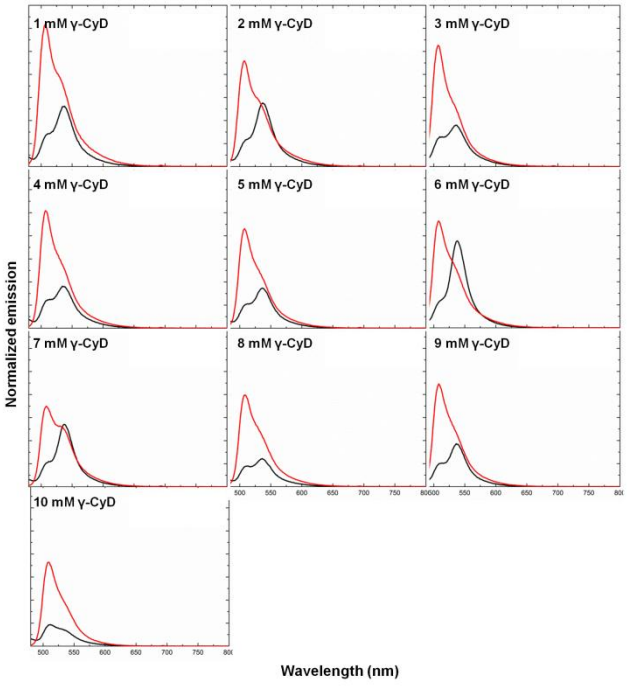
ZOOMED-IN SUMMARY FIGURES FOR ALL ANALYTE-FLUOROPHORE COMBINATIONS

Figures are at the maximum fluorophore concentration unless otherwise noted. The black line represents the fluorescence from excitation of the analyte, and the red line represents the fluorescence from direct fluorophore excitation.

Anthracene (**1**) – Rhodamine (**8**):



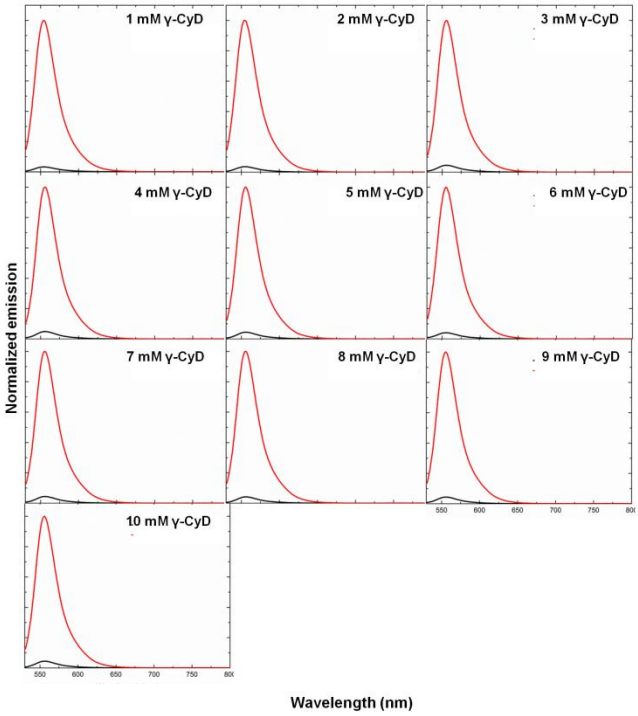
Anthracene (**1**) – BODIPY (**9**):



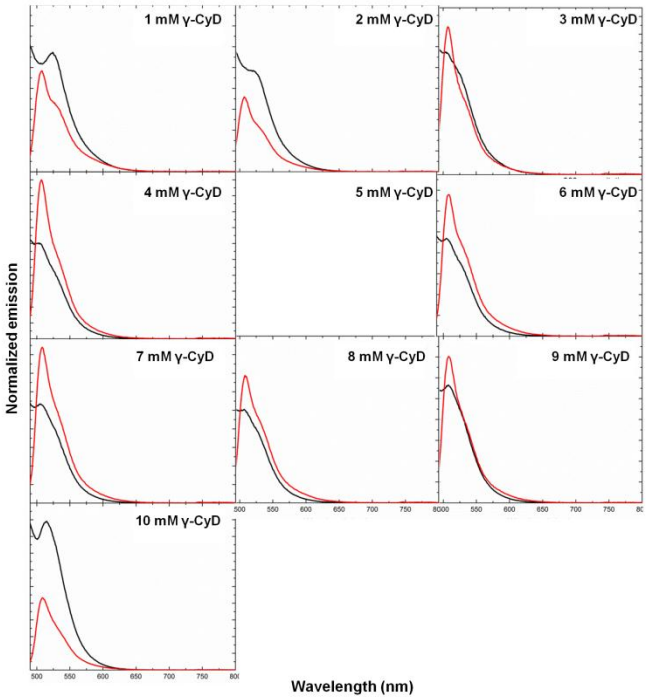
Anthracene (**1**) – Squaraine (**10**):

Refer to Reference 12: T. Mako, P. Marks, N. Cook and M. Levine, *Supramol. Chem.*, 2012, **24**, 743.

Pyrene (**2**) – Rhodamine (**8**):



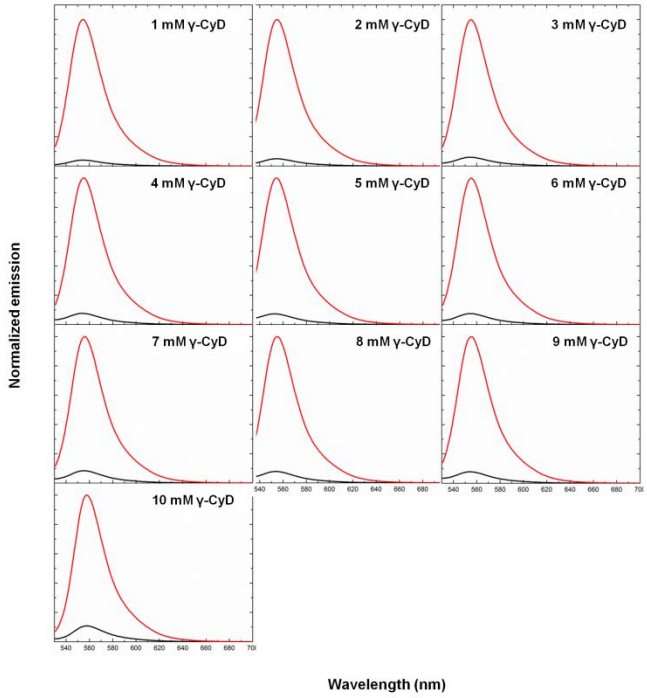
Pyrene (**2**) – BODIPY (**9**):



Pyrene (**2**) – Squaraine (**10**):

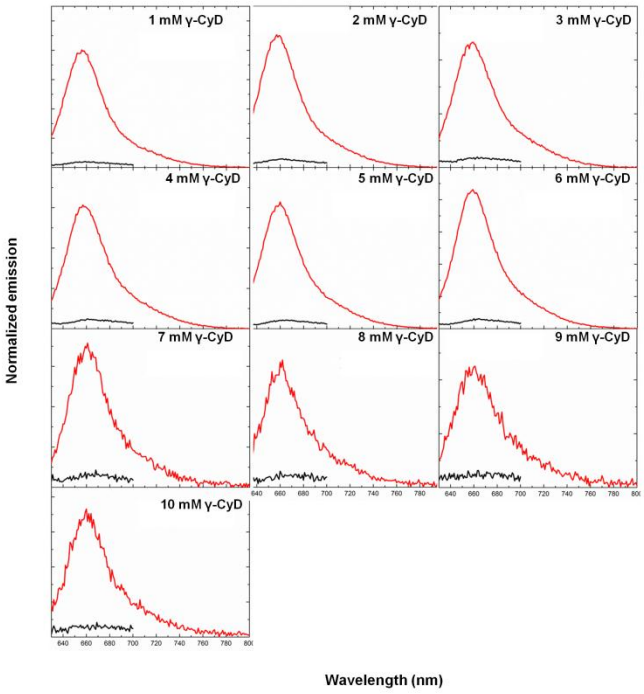
Refer to Reference 12: T. Mako, P. Marks, N. Cook and M. Levine, *Supramol. Chem.*, 2012, **24**, 743.

Benzo[*a*]pyrene (**3**) – Rhodamine (**8**):

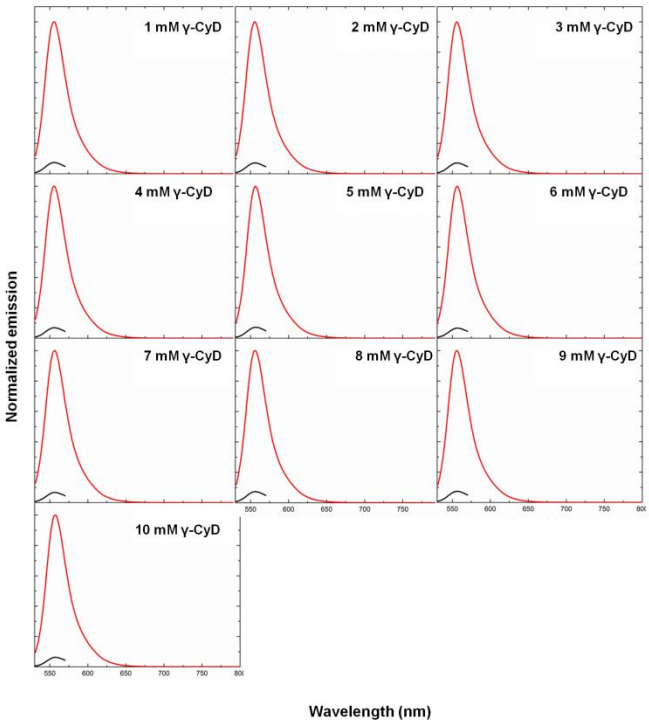


Benzo[*a*]pyrene (**3**) – BODIPY (**9**): Excessive overlap between the benzo[*a*]pyrene excimer emission and the BODIPY emission.

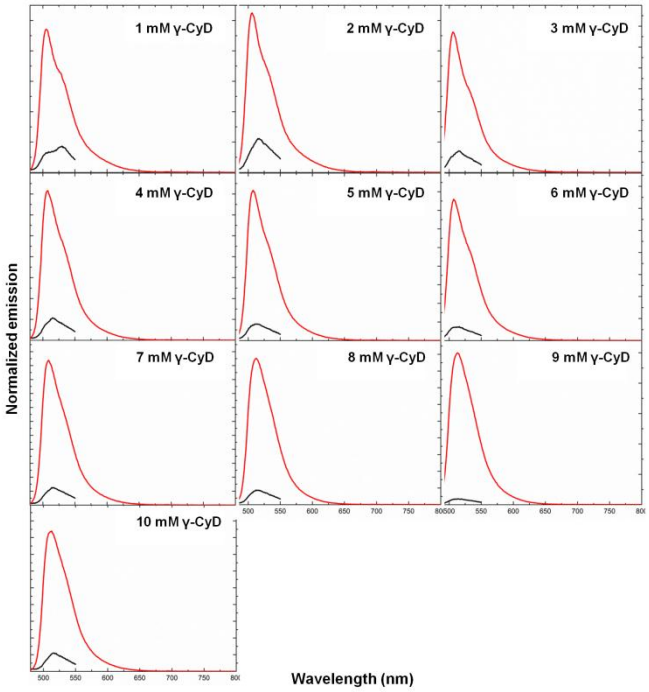
Benzo[*a*]pyrene (**3**) – Squaraine (**10**):



Phenanthrene (**4**) – Rhodamine (**8**):

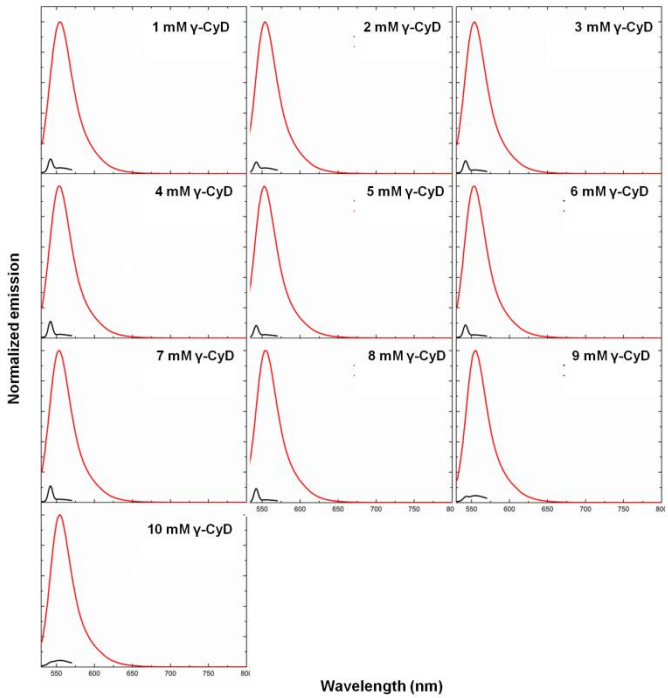


Phenanthrene (**4**) – BODIPY (**9**):

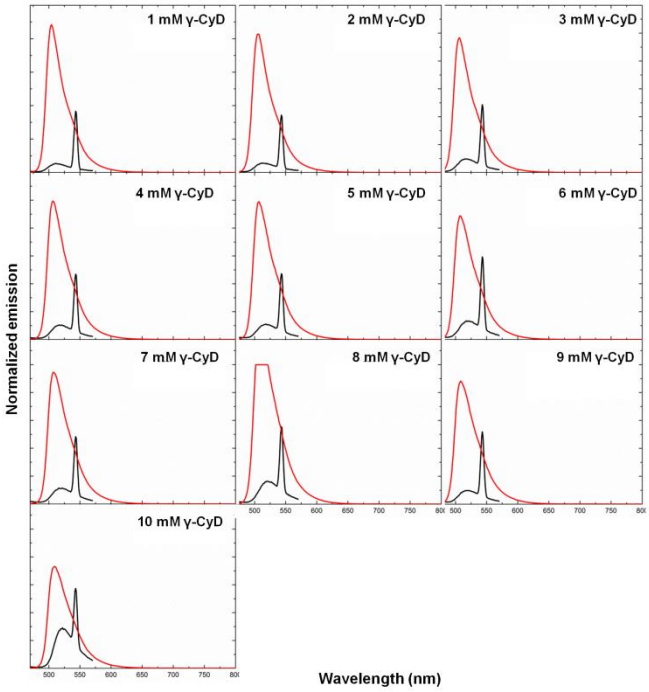


Phenanthrene (**4**) – Squaraine (**10**): Preliminary results indicate no energy transfer.

Fluorene (**5**) – Rhodamine (**8**):

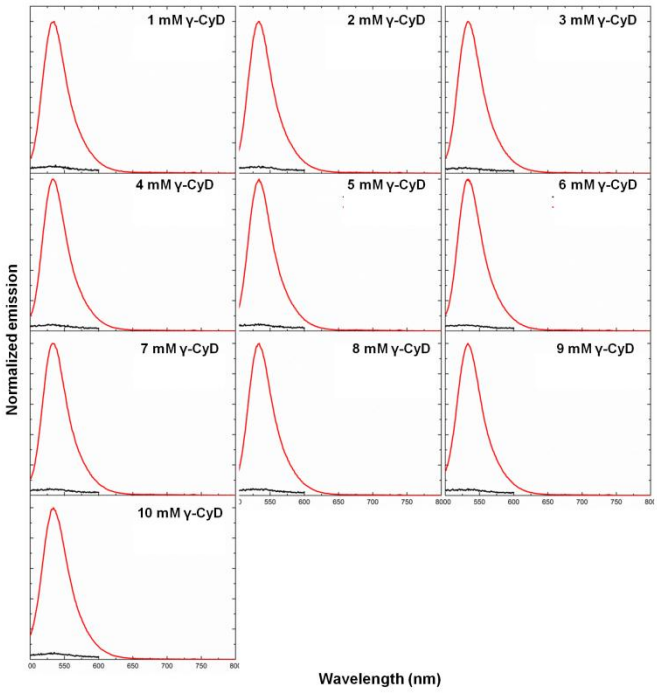


Fluorene (**5**) – BODIPY (**9**):

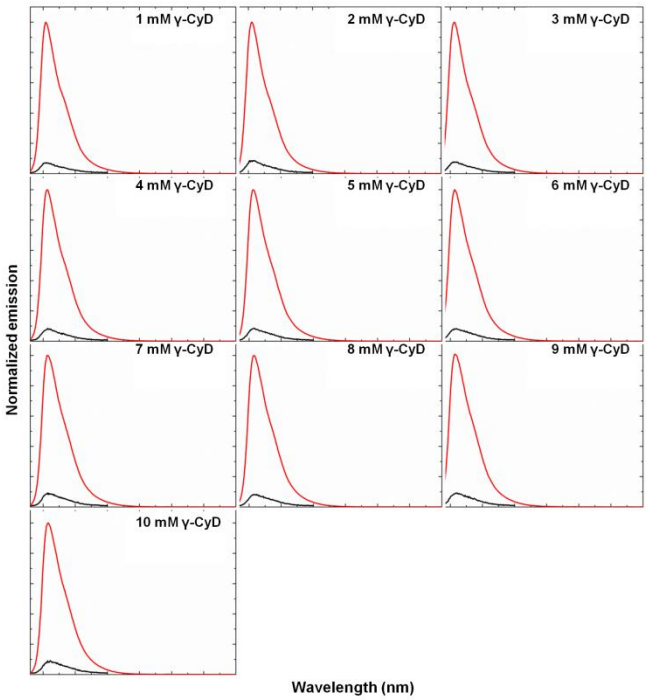


Fluorene (**5**) – Squaraine (**10**): Preliminary results indicate no energy transfer

4,4'-Dichlorobiphenyl (**6**) – Rhodamine (**8**):



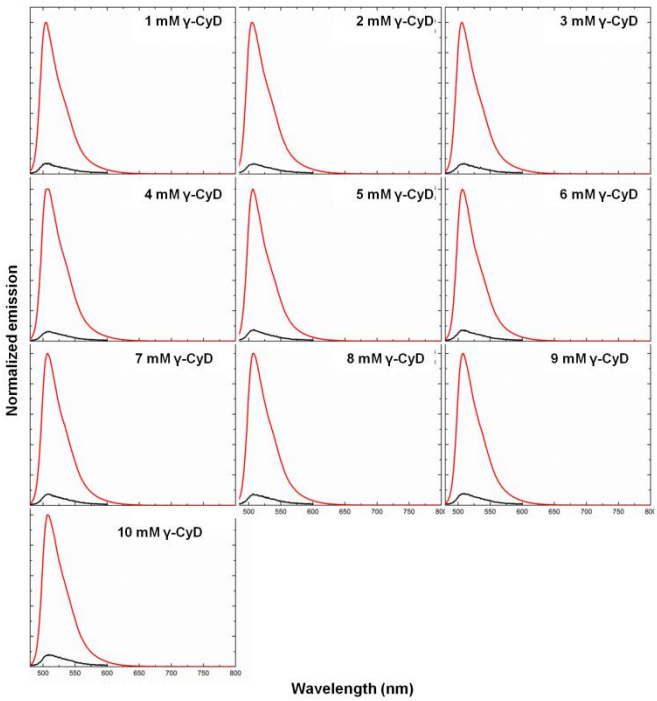
4,4'-Dichlorobiphenyl (**6**) – BODIPY (**9**):



4,4'-Dichlorobiphenyl (**6**) – Squaraine (**10**): Preliminary results indicate no energy transfer is observed.

PCB 29 (**7**) – Rhodamine (**8**): Preliminary results indicate no energy transfer is observed.

PCB 29 (**7**) – BODIPY (**9**):



PCB 29 (**7**) – Squaraine (**10**): Preliminary results indicate no energy transfer is observed.

[S1] J. L. Shepherd, A. Kell, E. Chung, C. W. Sinclar, M. S. Workentin and D. Bizzotto, *J. Am. Chem. Soc.*, 2004, **126**, 8329.

[S2] P. T. Snee, R. C. Somers, G. Nair, J. P. Zimmer, M. G. Bawendi and D. G. Nocera, *J. Am. Chem. Soc.*, 2006, **128**, 13320; Y. G. Isgor and E. U. Akkaya, *Tetrahedron Lett.*, 1997, **38**, 7417.