Electronic Supplementary Material (ESI) for Analyst.
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Electronic Supplementary Information for

Rapid and Efficient Pesticide Detection via Cyclodextrin-Promoted Energy Transfer

Nicole Serio, John Roque, Andrew Badwal, and Mindy Levine

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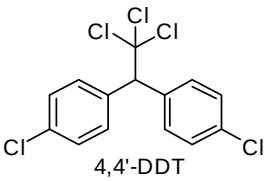
MATERIALS AND METHODS

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, with 1.5 nm excitation slit width and 1.5 nm emission slit width. All spectra were integrated vs. wavenumber using OriginPro software.

The apple juice is Simply Apple™ brand and was purchased from a local grocery store.

ANALYTE DETAILS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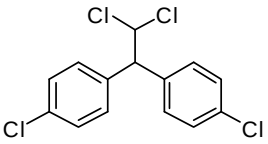
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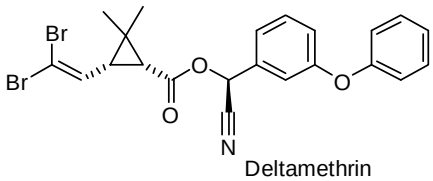
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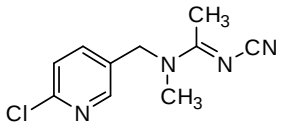
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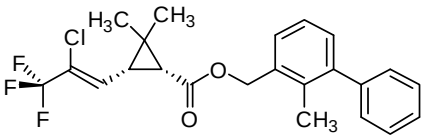
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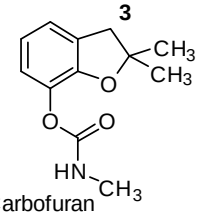
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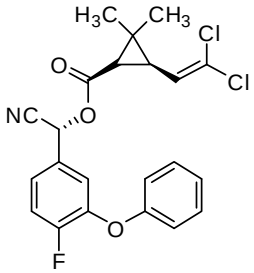
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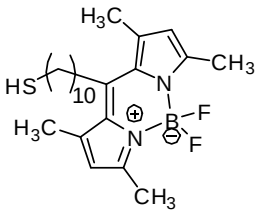
BODIPY
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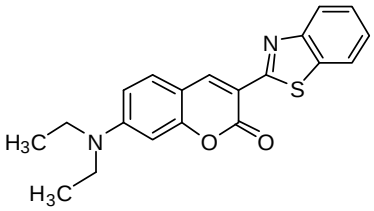
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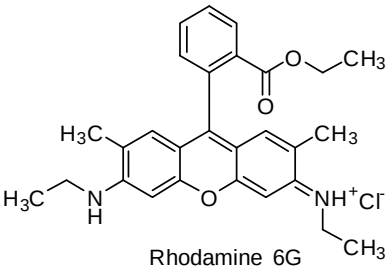

Rhodamine 6G
10

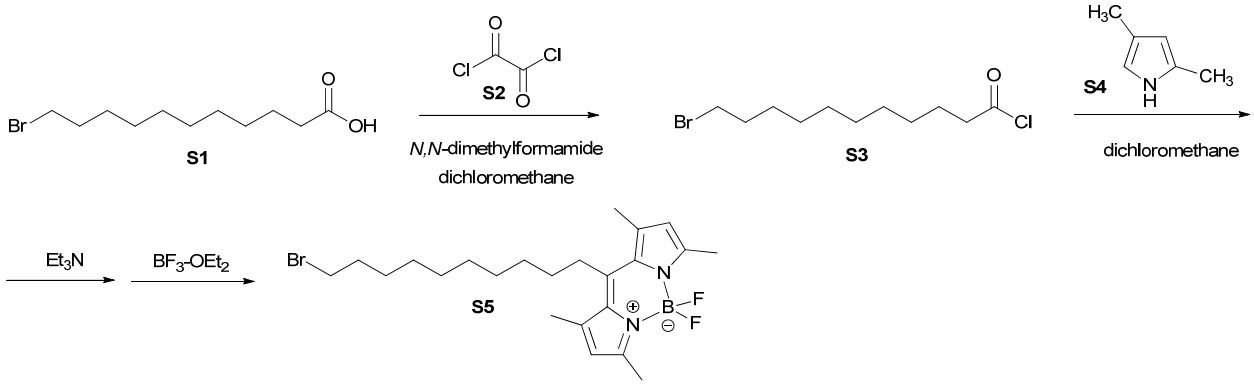
Analyte **1**: Excitation at 420 nm; emission from 430-700 nm
Analyte **2**: Excitation at 420 nm; emission from 430-700 nm
Analyte **3**: Excitation at 310 nm; emission from 320-700 nm
Analyte **4**: Excitation at 320 nm; emission from 330-700 nm
Analyte **5**: Excitation at 290 nm; emission from 300-700 nm
Analyte **6**: Excitation at 250 nm; emission from 260-700 nm
Analyte **7**: Excitation at 290 nm; emission from 300-700 nm
Fluorophore **8**: Excitation at 460 nm; emission from 470-800 nm
Fluorophore **9**: Excitation at 420 nm; emission from 430-800 nm
Fluorophore **10**: Excitation at 490 nm; emission from 500-800 nm

SYNTHESIS OF FLUOROPHORE 8

The synthesis of BODIPY **8** was performed according to literature procedures:

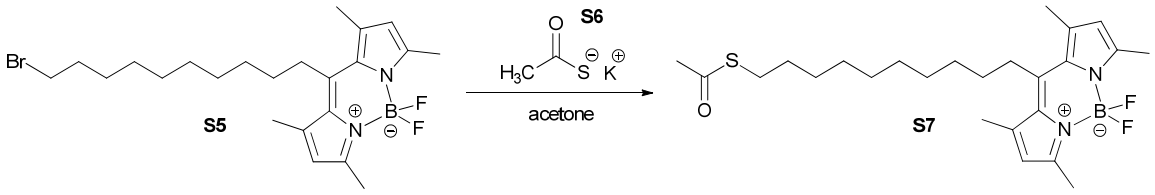
Shepherd, J. L.; Kell, A.; Chung, E.; Sinclair, C. W.; Workentin, M. S.; Bizzotto, D. *J. Am. Chem. Soc.* **2004**, *126*, 8329-8335.

Reaction 1:



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).

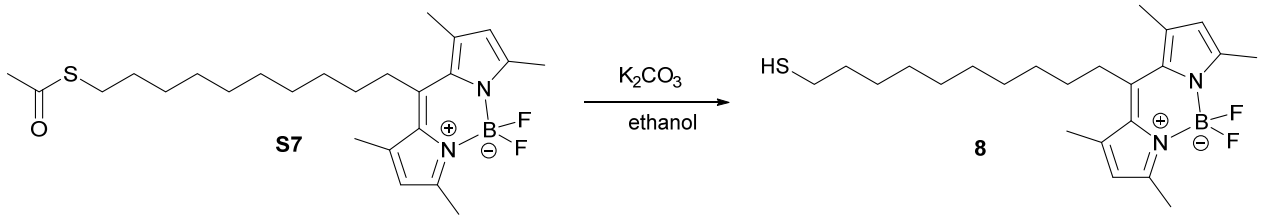
Reaction 2:



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 **8** in 76% yield (674 mg).

GENERAL PROCEDURE FOR ENERGY TRANSFER EXPERIMENTS

100 μL of fluorophores **8-10** (0.1 mg/mL in THF) was added to a 10 mM solution of γ-cyclodextrin in phosphate buffered saline (PBS), buffered at pH 7.4. The solution was excited at the excitation wavelength of the analyte and at the fluorophore excitation wavelength. Then, 20 μL of analytes **1-7** (1 mg/mL in THF) was added, and the solution was again excited at the analyte excitation wavelength and fluorophore excitation wavelength. The energy transfer efficiencies were calculated as in Equation 1:

Energy transfer efficiency = I_{DA}/I_A x 100% (Eq. 1)

Where I_{DA} is the integration of the fluorophore from analyte excitation and I_A is the integrated fluorophore emission from direct excitation.

Control experiments were also conducted in which the 10 mM γ-cyclodextrin solution was replaced with a 0 mM solution, and the same procedure was followed.

All experiments were repeated 4 times, and the values reported are averages of the results.

GENERAL PROCEDURE FOR LOD 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 the wavelength listing on page 6) and the fluorescence emission spectrum was recorded. Six 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. Six 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 calibration curves were generated with the analyte concentration on the X-axis (in μM) and the integrated fluorophore emission on the Y-axis. The curve was then fitted to a straight line and an equation for the line was determined.

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

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

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

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

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

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

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

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

SUMMARY TABLES

IN PHOSPHATE BUFFERED SALINE:

Doped energy transfer efficiencies:

analyte	fluorophore	10 mM γ-CD	0 mM γ-CD
1	**8**	8.2 ± 0.1	8.7 ± 0.0
2	**8**	9.2 ± 0.1	8.7 ± 0.0
3	**8**	22.2 ± 0.1	17.7 ± 0.1
4	**8**	43.3 ± 1.0	27.5 ± 0.1
5	**8**	54.9 ± 1.2	25.4 ± 0.6
6	**8**	a	a
7	**8**	48.7 ± 1.1	39.6 ± 0.6
1	**9**	a	a
2	**9**	a	a
3	**9**	56.5 ± 0.9	46.4 ± 0.8
4	**9**	53.6 ± 0.2	82.6 ± 1.1
5	**9**	51.6 ± 0.4	65.4 ± 1.4
6	**9**	a	a
7	**9**	44.8 ± 1.2	76.6 ± 2.7
1	**10**	4.0 ± 0.0	4.0 ± 0.0
2	**10**	5.0 ± 0.0	4.2 ± 0.0
3	**10**	9.2 ± 0.0	8.9 ± 0.3
4	**10**	5.3 ± 0.0	5.5 ± 0.0
5	**10**	8.7 ± 0.0	11.9 ± 0.0
6	**10**	11.2 ± 0.1	13.6 ± 0.1
7	**10**	8.9 ± 0.0	16.1 ± 0.1

Undoped energy transfer efficiencies:

Analyte	Fluorophore	10 mM γ-CD	0 mM γ-CD
1	**8**	8.9 ± 0.2	8.7 ± 0.1
2	**8**	9.0 ± 0.1	8.8 ± 0.1
3	**8**	23.3 ± 0.1	17.5 ± 0.1
4	**8**	37.8 ± 0.7	31.6 ± 0.6
5	**8**	51.7 ± 1.0	81.1 ± 1.9
6	**8**	154.6 ± 2.2	148.6 ± 3.7
7	**8**	51.7 ± 1.0	49.9 ± 1.1
1	**9**	*a*	*a*
2	**9**	*a*	*a*
3	**9**	52.1 ± 1.0	49.1 ± 2.0
4	**9**	53.5 ± 0.3	48.0 ± 2.4
5	**9**	59.9 ± 0.4	72.4 ± 2.5
6	**9**	121.1 ± 1.1	191.9 ± 2.7
7	**9**	59.9 ± 0.3	109.0 ± 1.7
1	**10**	4.0 ± 0.0	3.9 ± 0.0
2	**10**	4.9 ± 0.0	4.1 ± 0.0
3	**10**	9.1 ± 0.1	8.7 ± 0.0
4	**10**	5.2 ± 0.0	5.5 ± 0.0
5	**10**	8.9 ± 0.0	11.8 ± 0.1
6	**10**	11.3 ± 0.0	13.4 ± 0.1
7	**10**	8.9 ± 0.1	15.5 ± 0.4

IN APPLE JUICE:

Doped energy transfer efficiencies:

analyte	fluorophore	10 mM γ-CD	0 mM γ-CD
1	**8**	25.0 ± 0.4	28.7 ± 0.1
2	**8**	24.3 ± 0.2	25.7 ± 0.1
3	**8**	*a*	*a*
4	**8**	*a*	*a*
5	**8**	*a*	*a*
6	**8**	*a*	*a*
7	**8**	*a*	*a*
1	**9**	*b*	*b*
2	**9**	*b*	*b*
3	**9**	*a*	*a*
4	**9**	*a*	*a*
5	**9**	*a*	*a*
6	**9**	*a*	*a*
7	**9**	*a*	*a*
1	**10**	4.8 ± 0.1	0.8 ± 0.0
2	**10**	4.9 ± 0.0	0.9 ± 0.0
3	**10**	*a*	*a*
4	**10**	*a*	*a*
5	**10**	*a*	*a*
6	**10**	*a*	*a*
7	**10**	*a*	*a*

LIMIT OF DETECTION IN APPLE JUICE SUMMARY TABLE:

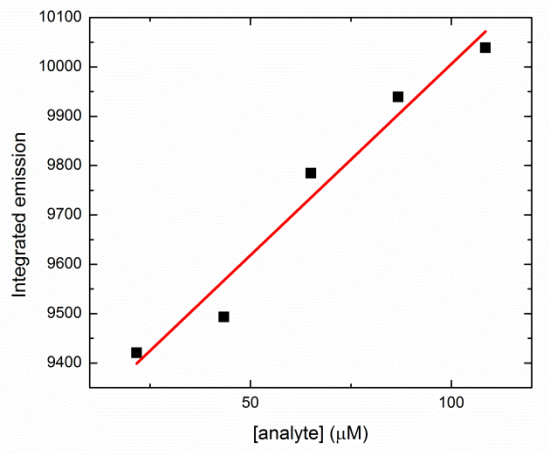
Analyte	Dye	LOD	LOQ	Equation	R^2
1	**8**	14.2	50.4	y = 7.7509x + 9230.7	0.9634
1	**9**	65.5	100.7	y = 8.398x + 12993	0.7822
1	**10**	6.4	11.5	y = 250.99x + 55117	0.9954
2	**8**	2.1	45.1	y = 21.63x + 11223	0.9785
2	**9**	13.3	65.1	y = 8.4992x + 13852	0.9508
2	**10**	5.5	6.8	y = 238.48x + 57288	0.9947

Other analyte-fluorophore combinations led to no real energy transfer peak.

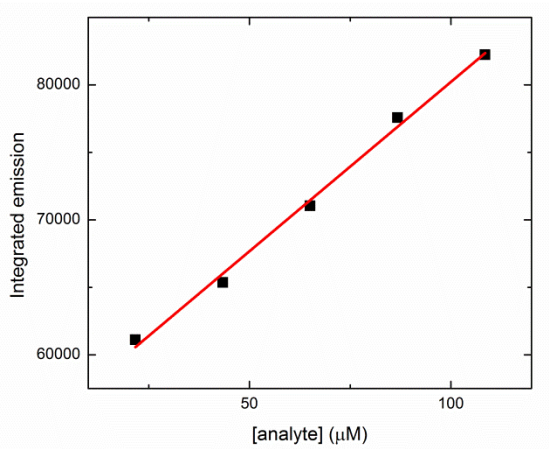
SUMMARY FIGURES

LIMIT OF DETECTION FIGURES

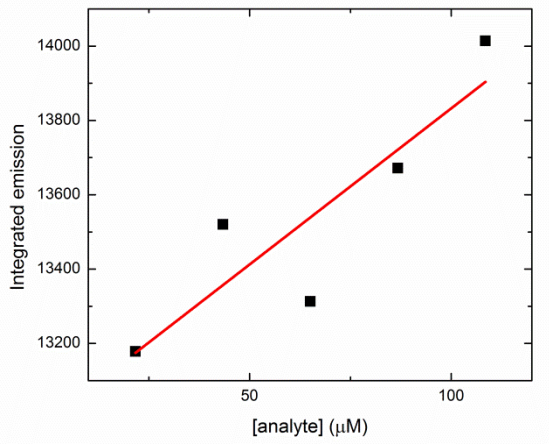
Analyte **1** – Fluorophore **8**



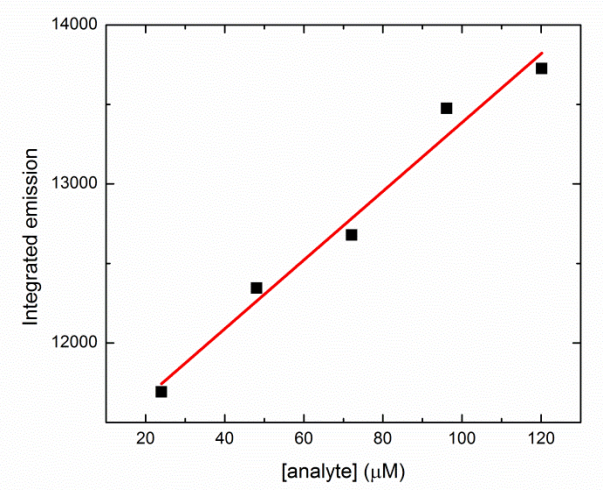
Analyte **1** – Fluorophore **9**



Analyte **1** – Fluorophore **10**



Analyte **2** – Fluorophore **8**



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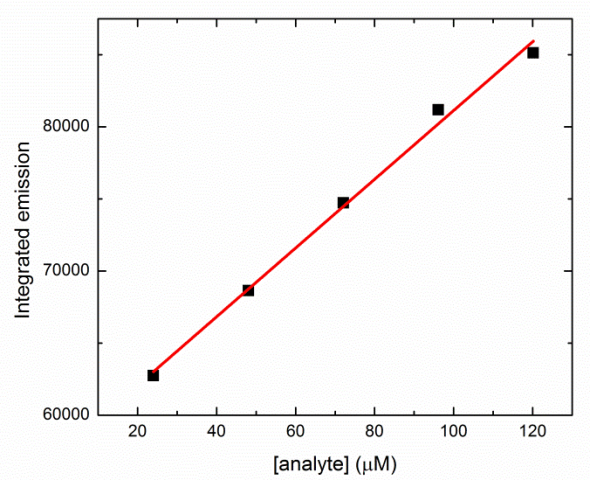
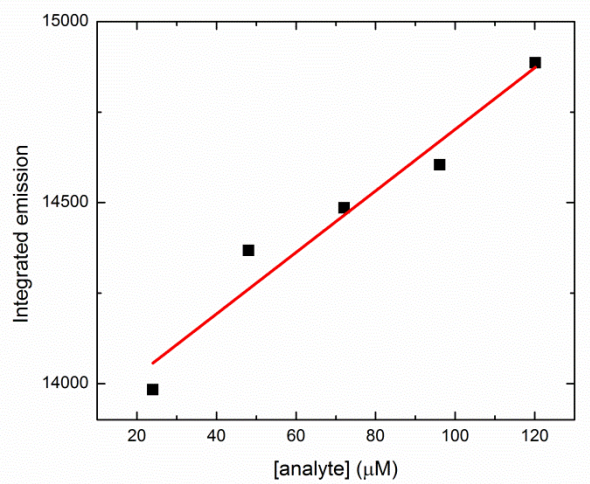


Analyte **2** – Fluorophore **10**

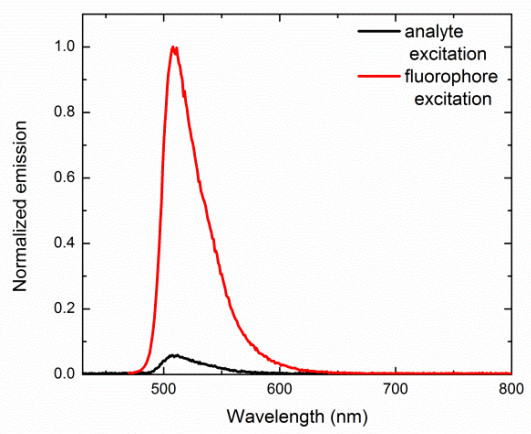


UNDOPED SOLUTIONS ENERGY TRANSFER

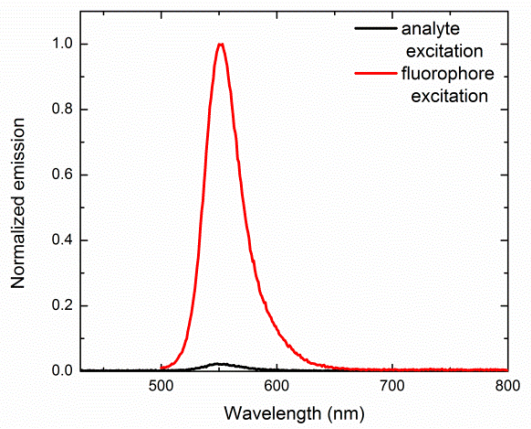
Fluorophore excitation at the analyte wavelength, without analyte added, compared to fluorophore excitation at the fluorophore wavelength.

IN 10 MM γ-CYCLODEXTRIN IN PHOSPHATE BUFFERED SALINE:

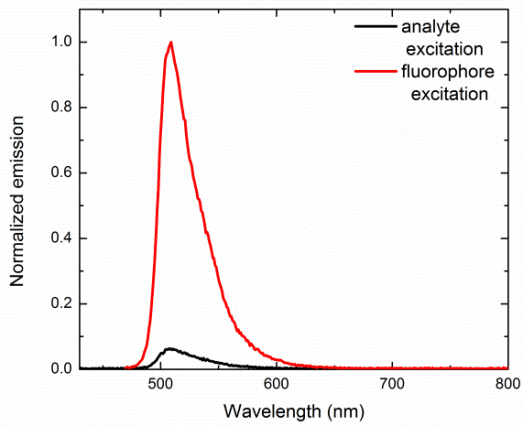
Analyte **1** – Fluorophore **8**



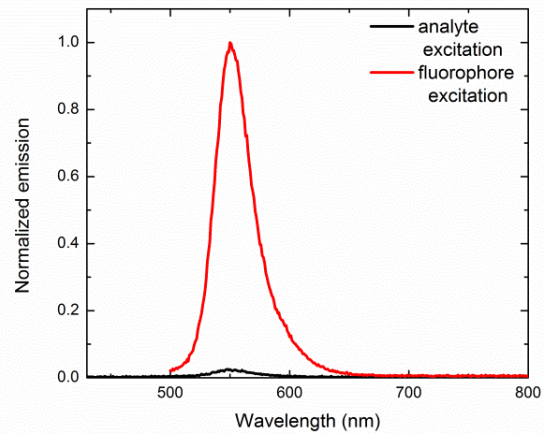
Analyte **1** – Fluorophore **10**



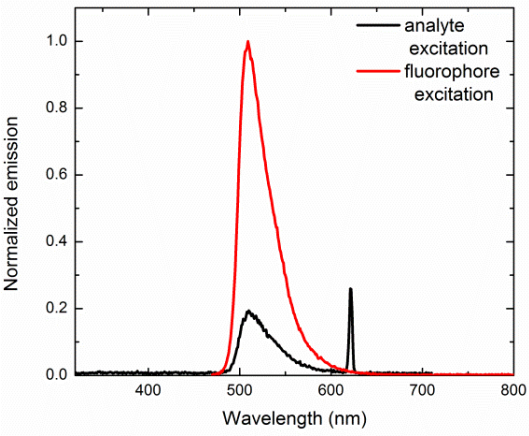
Analyte **2** – Fluorophore **8**



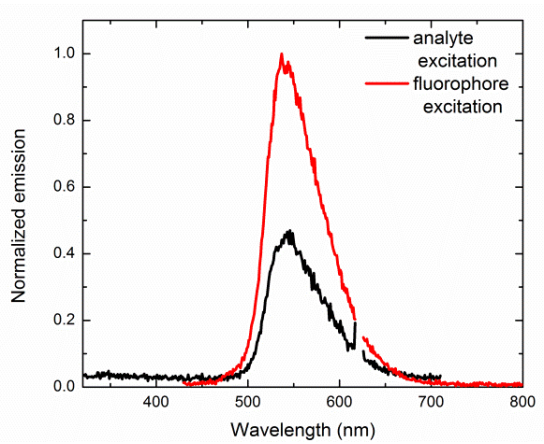
Analyte **2** – Fluorophore **10**



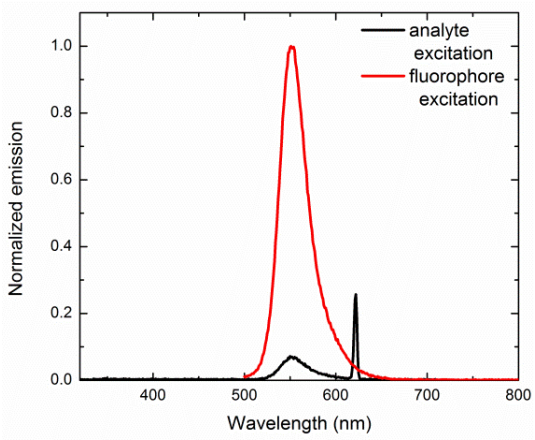
Analyte **3** – Fluorophore **8**



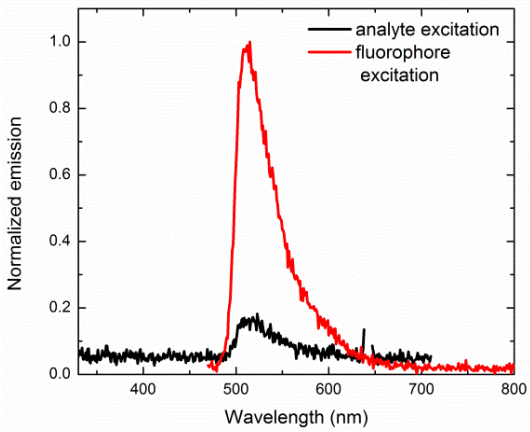
Analyte **3** – Fluorophore **9**



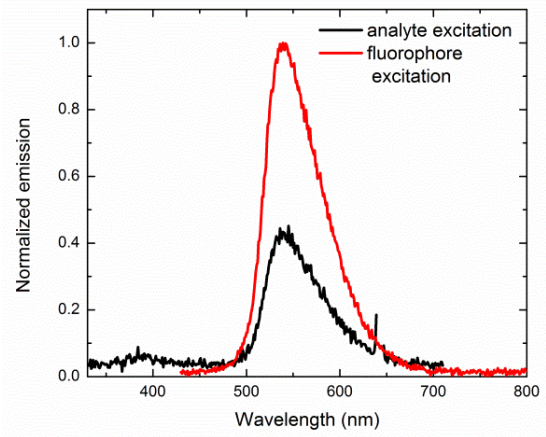
Analyte **3** – Fluorophore **10**



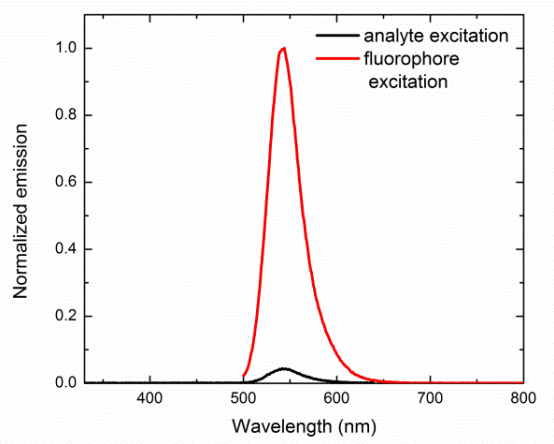
Analyte **4** – Fluorophore **8**



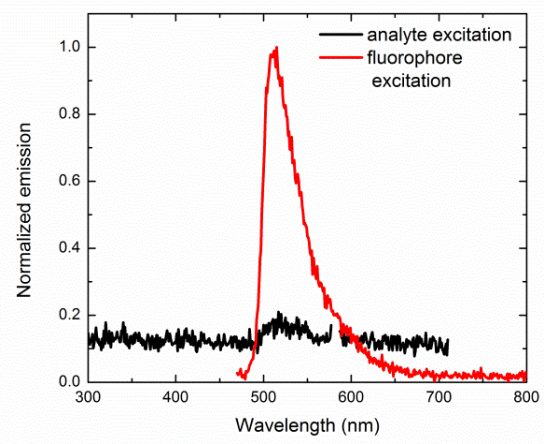
Analyte **4** – Fluorophore **9**



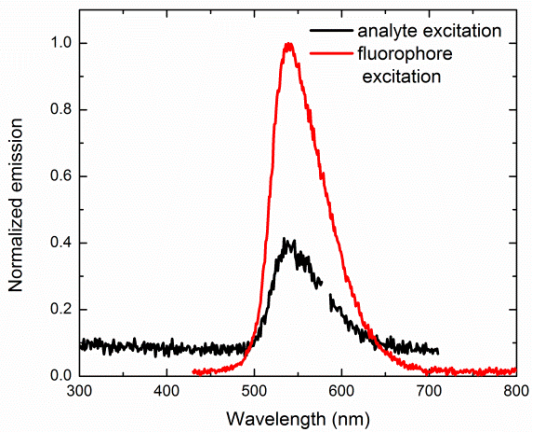
Analyte **4** – Fluorophore **10**



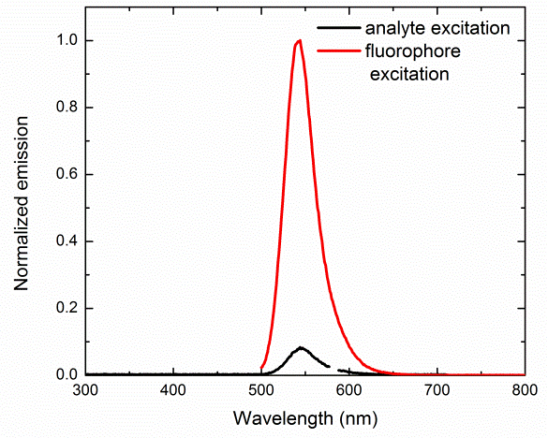
Analyte **5** – Fluorophore **8**



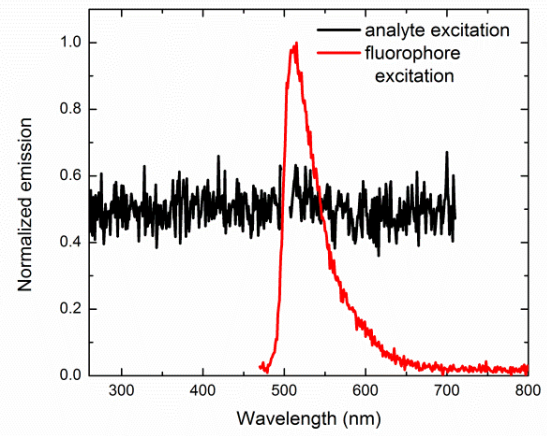
Analyte **5** – Fluorophore **9**



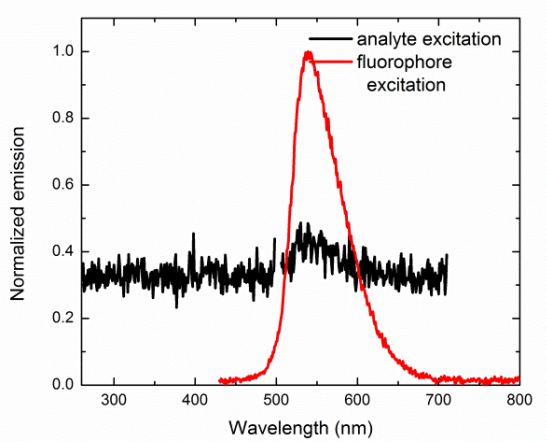
Analyte **5** – Fluorophore **10**



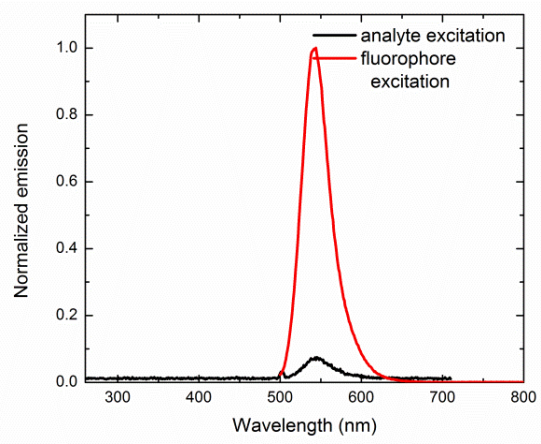
Analyte **6** – Fluorophore **8**



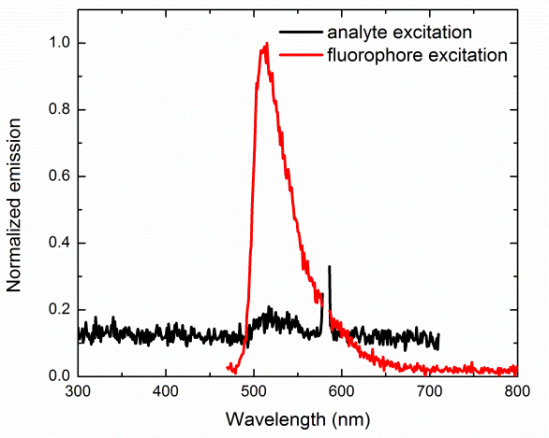
Analyte **6** – Fluorophore **9**



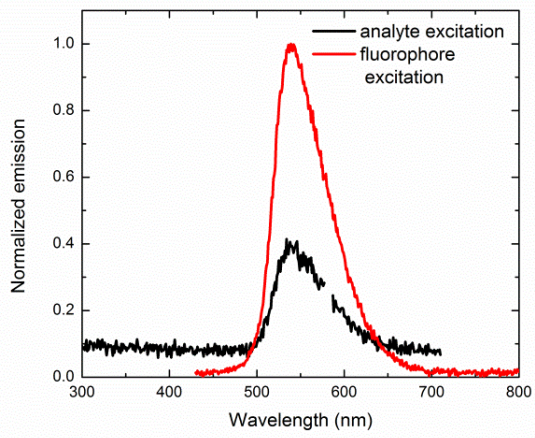
Analyte **6** – Fluorophore **10**



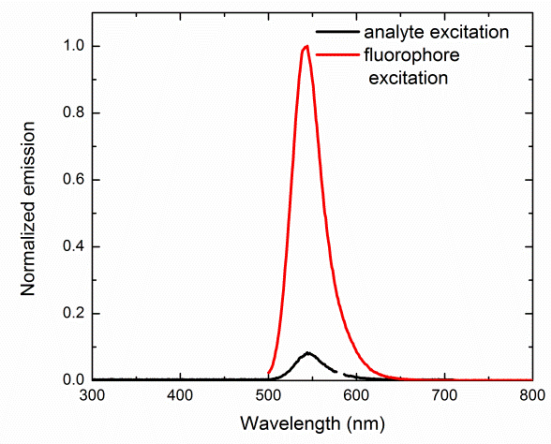
Analyte **7** – Fluorophore **8**



Analyte **7** – Fluorophore **9**

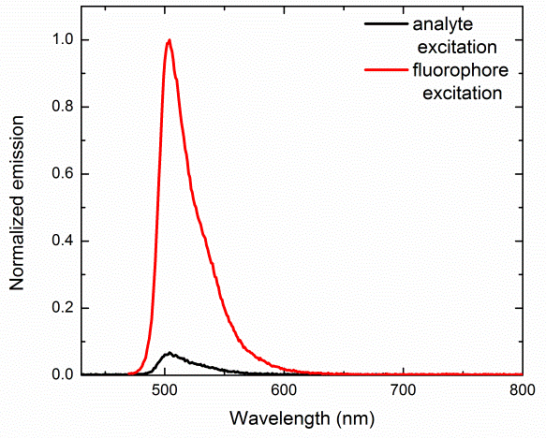


Analyte **7** – Fluorophore **10**

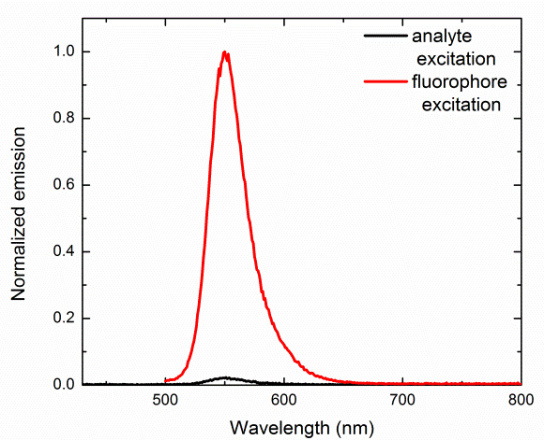


IN PHOSPHATE BUFFERED SALINE WITH 0 MM γ-CYCLODEXTRIN:

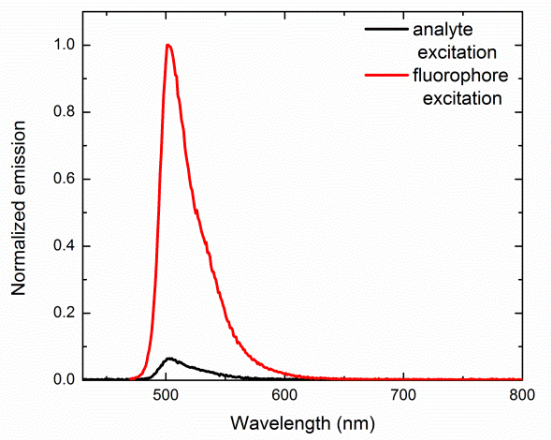
Analyte **1** – Fluorophore **8**



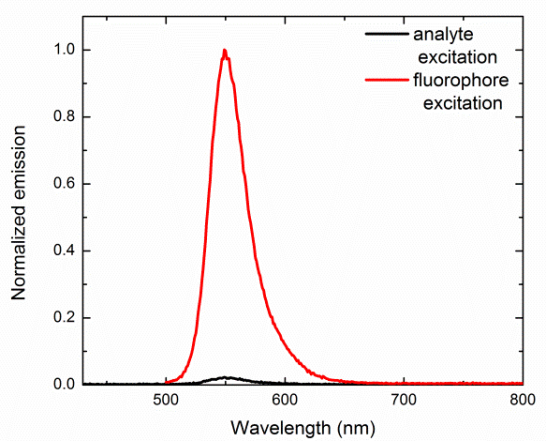
Analyte **1** – Fluorophore **10**



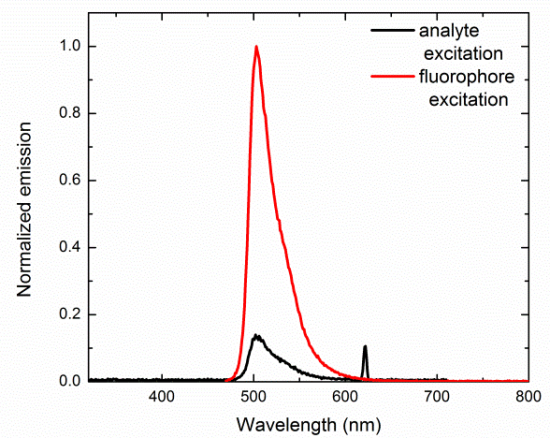
Analyte **2** – Fluorophore **8**



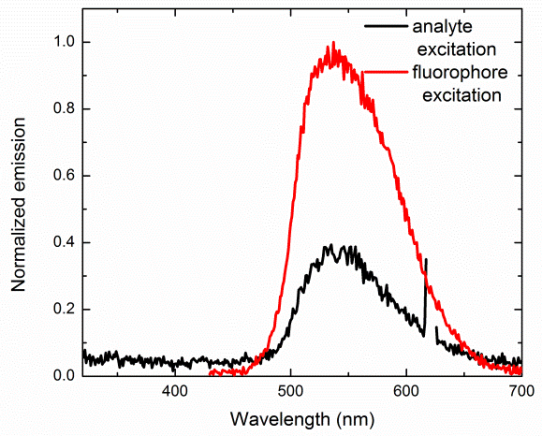
Analyte **2** – Fluorophore **10**



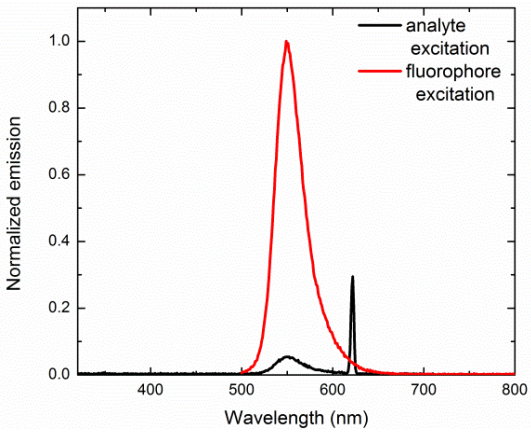
Analyte **3** – Fluorophore **8**



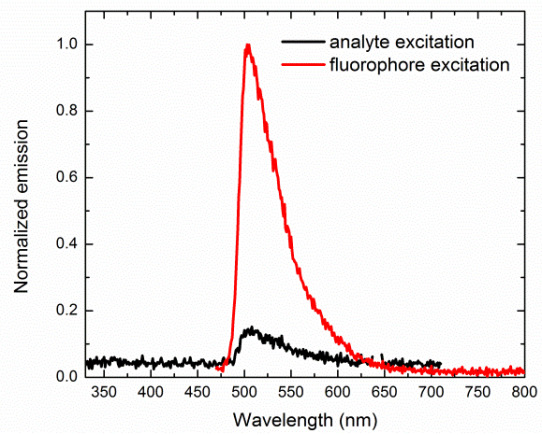
Analyte **3** – Fluorophore **9**



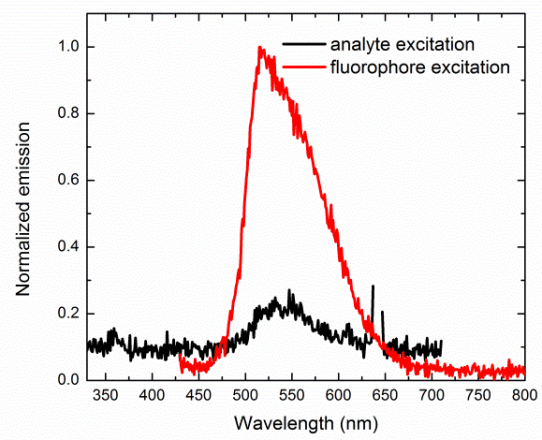
Analyte **3** – Fluorophore **10**



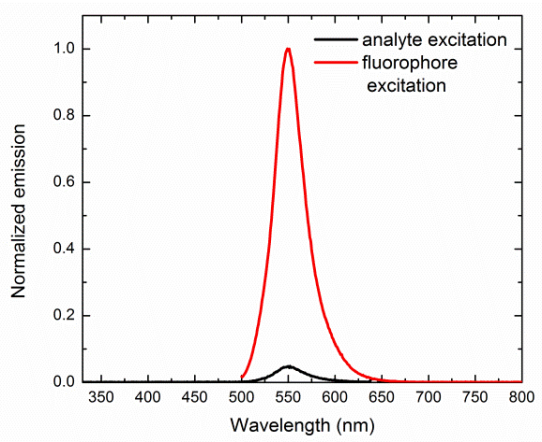
Analyte **4** – Fluorophore **8**



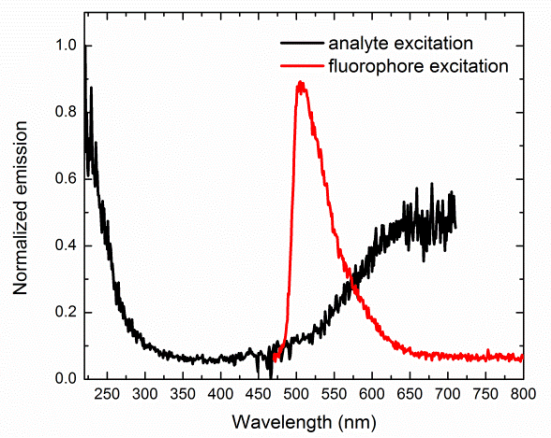
Analyte **4** – Fluorophore **9**



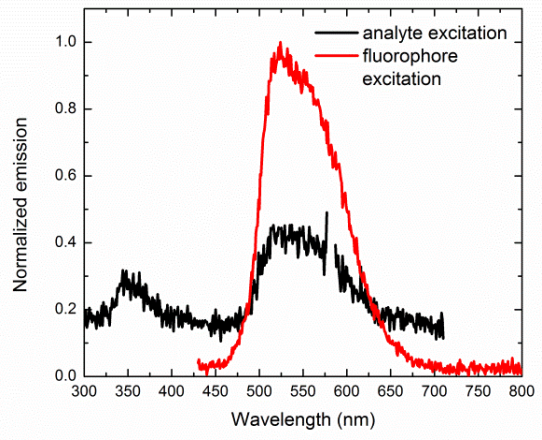
Analyte **4** – Fluorophore **10**



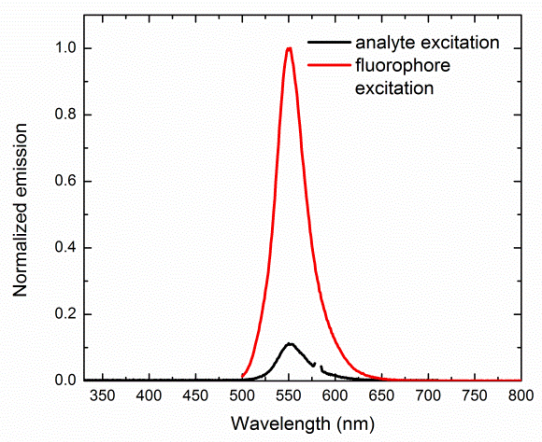
Analyte **5** – Fluorophore **8**



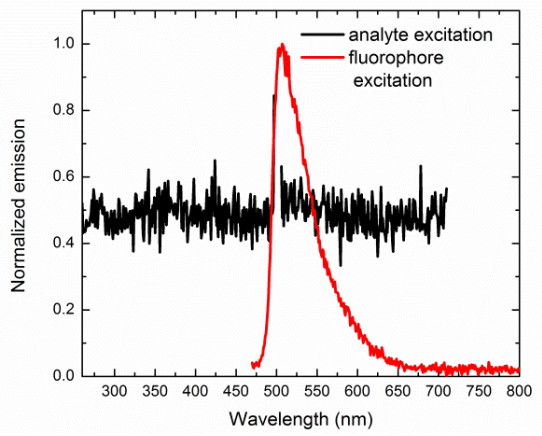
Analyte **5** – Fluorophore **9**



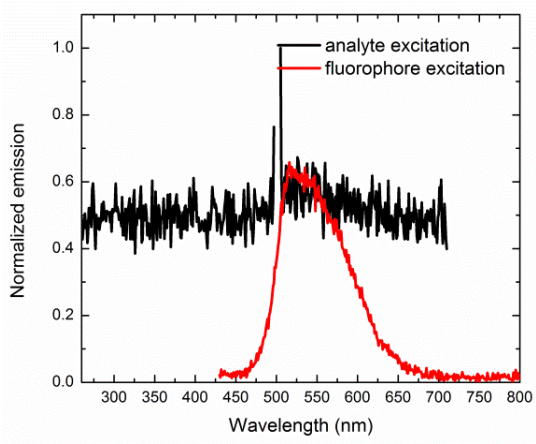
Analyte **5** – Fluorophore **10**



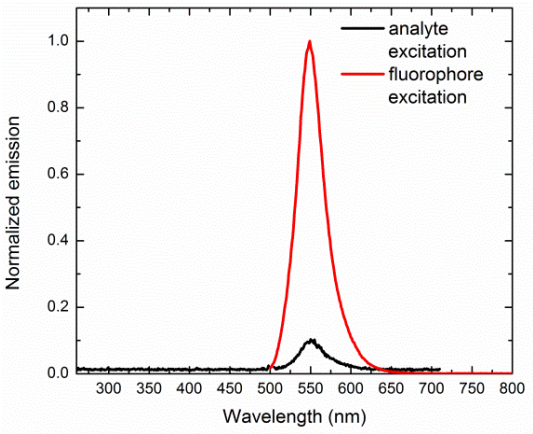
Analyte **6** – Fluorophore **8**



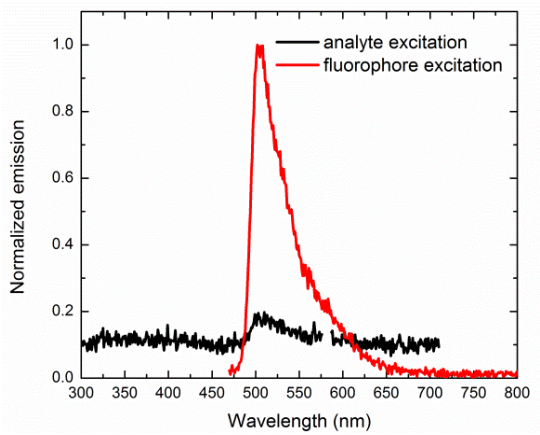
Analyte **6** – Fluorophore **9**



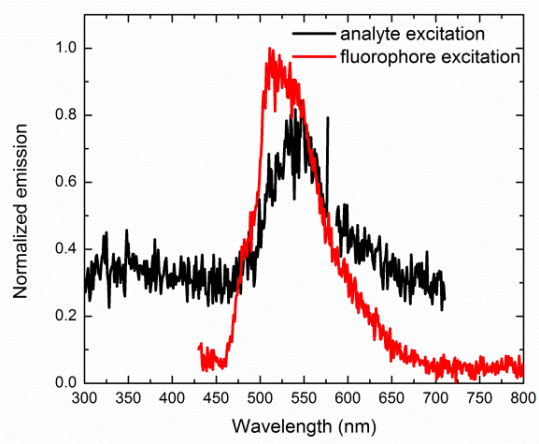
Analyte **6** – Fluorophore **10**



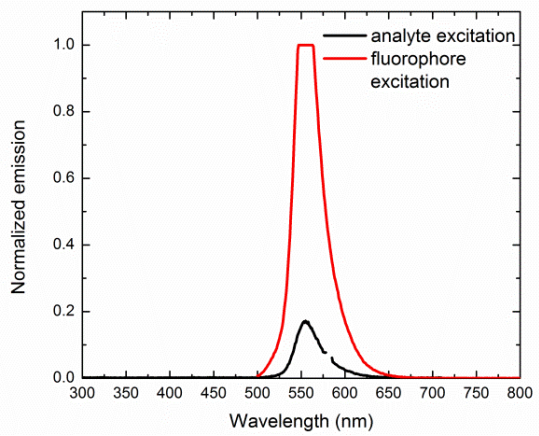
Analyte **7** – Fluorophore **8**



Analyte **7** – Fluorophore **9**

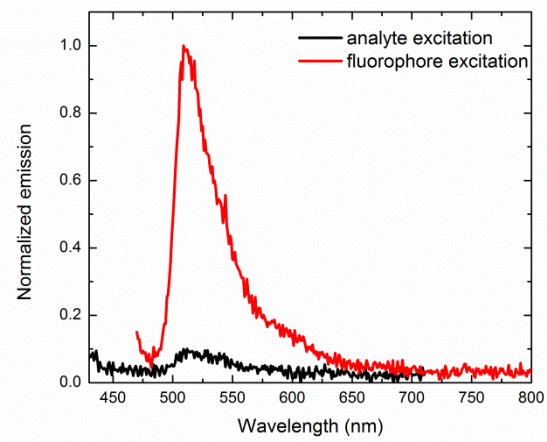


Analyte **7** – Fluorophore **10**

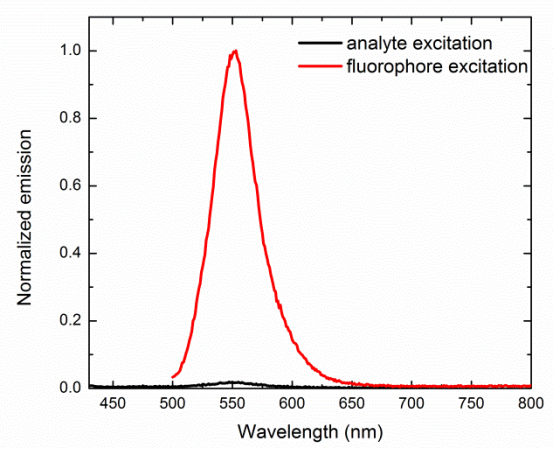


IN APPLE JUICE WITH 10 MM γ-CYCLODEXTRIN:

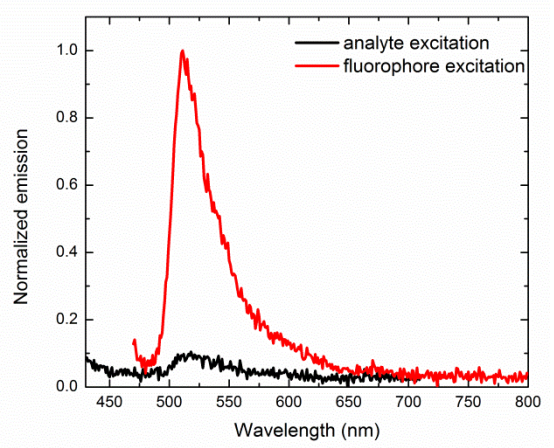
Analyte **1** – Fluorophore **8**



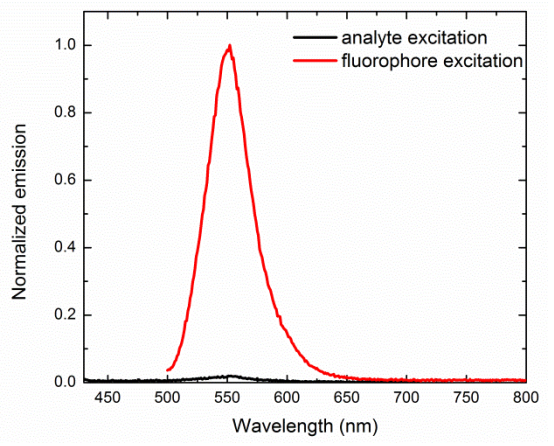
Analyte **1** – Fluorophore **10**



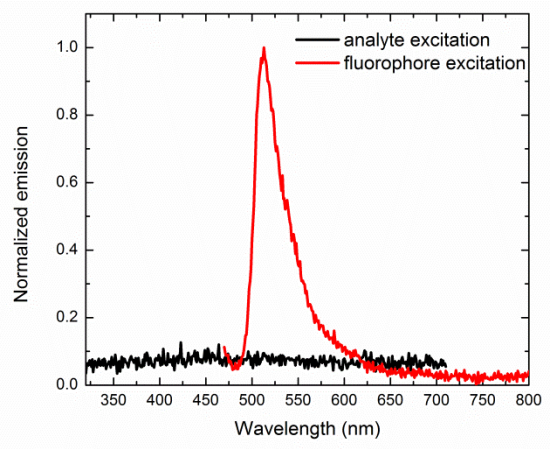
Analyte **2** – Fluorophore **8**



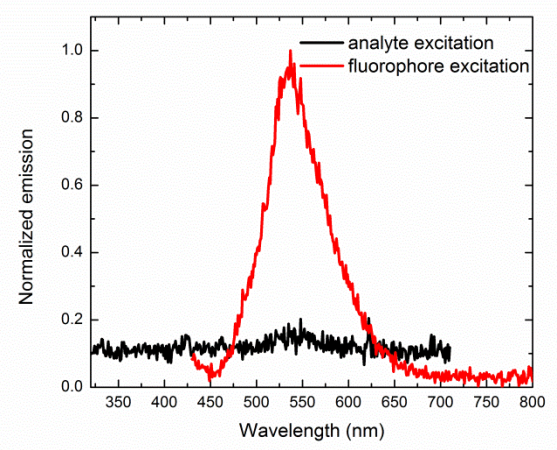
Analyte **2** – Fluorophore **10**



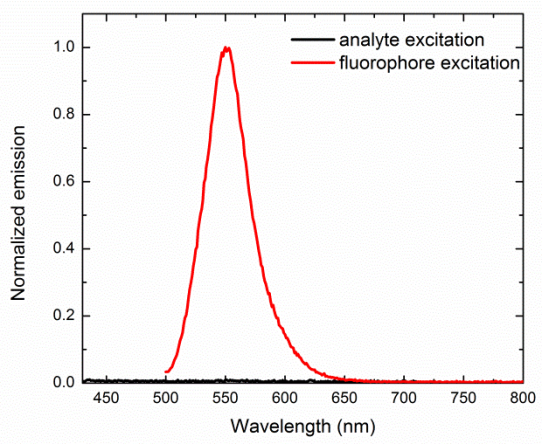
Analyte **3** – Fluorophore **8**



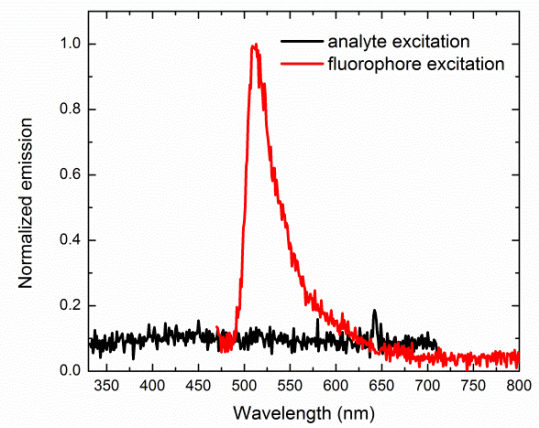
Analyte **3** – Fluorophore **9**



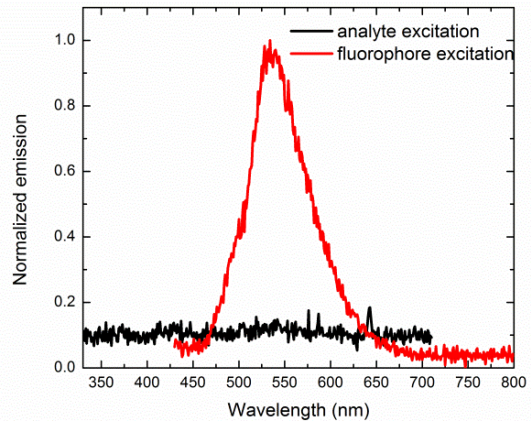
Analyte **3** – Fluorophore **10**



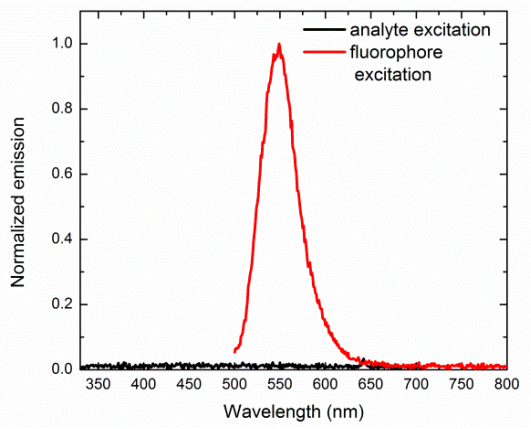
Analyte **4** – Fluorophore **8**



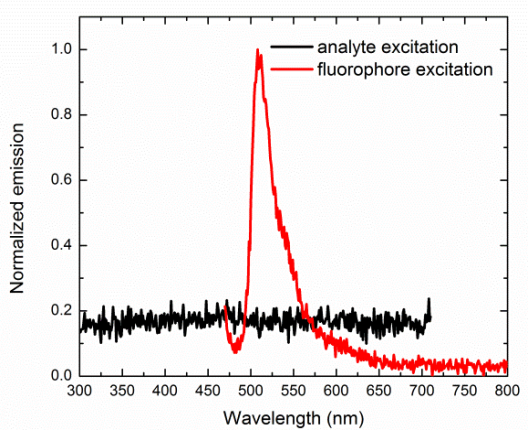
Analyte **4** – Fluorophore **9**



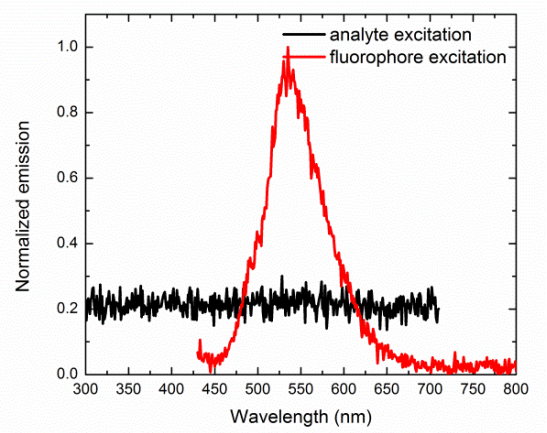
Analyte **4** – Fluorophore **10**



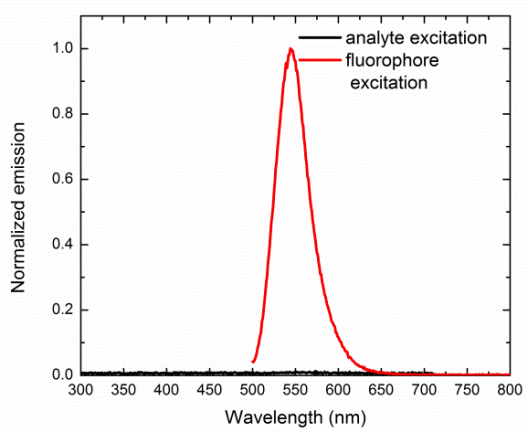
Analyte **5** – Fluorophore **8**



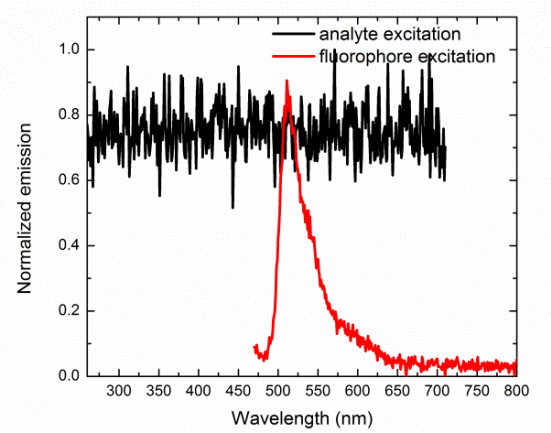
Analyte **5** – Fluorophore **9**



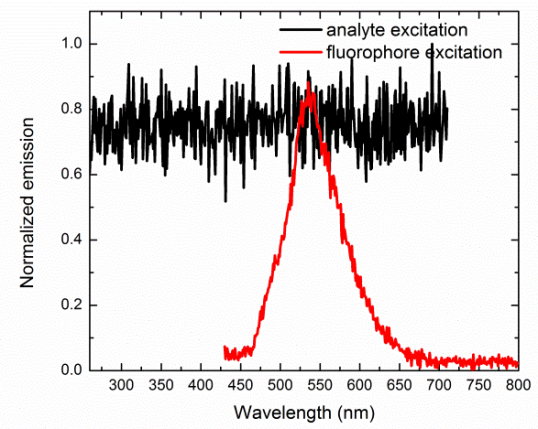
Analyte **5** – Fluorophore **10**



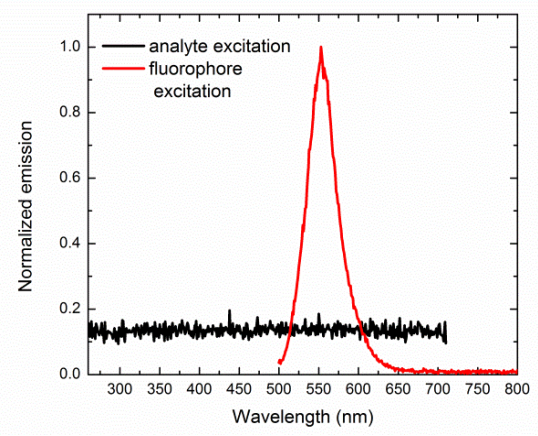
Analyte **6** – Fluorophore **8**



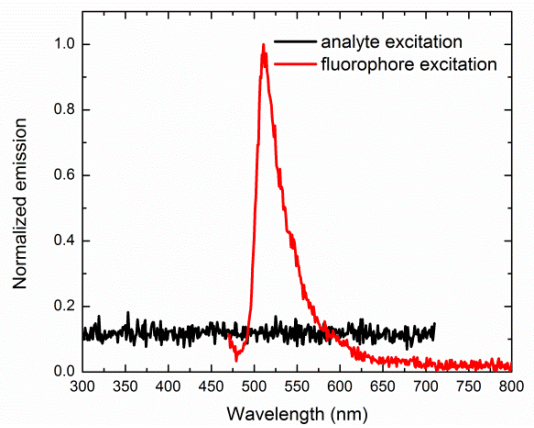
Analyte **6** – Fluorophore **9**



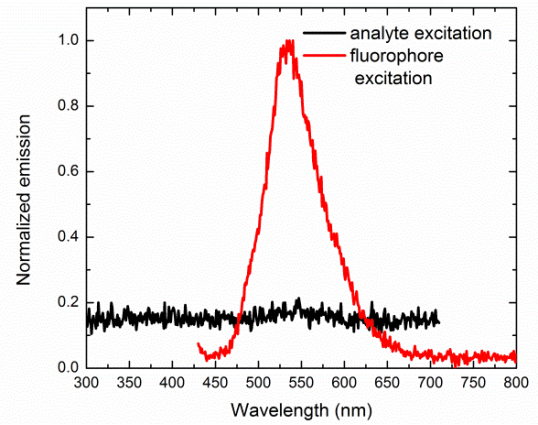
Analyte **6** – Fluorophore **10**



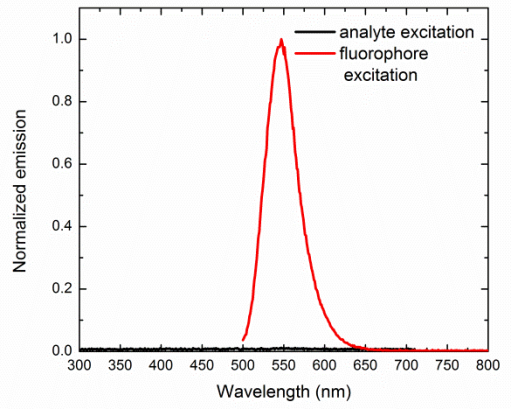
Analyte **7** – Fluorophore **8**



Analyte **7** – Fluorophore **9**

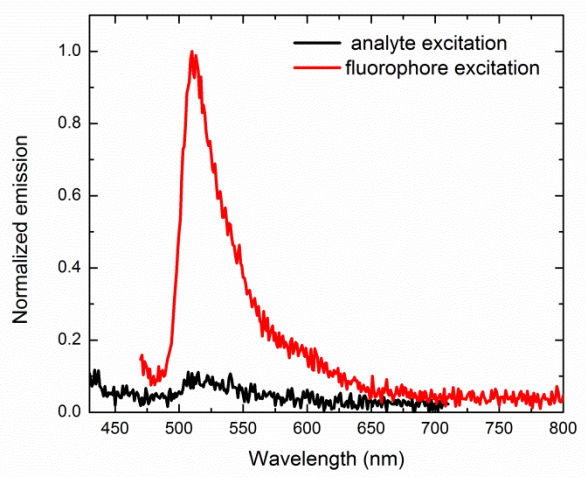


Analyte **7** – Fluorophore **10**

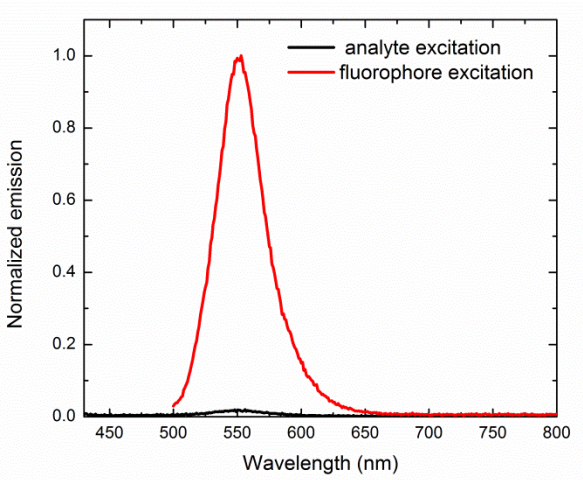


IN APPLE JUICE WITH 0 MM γ-CYCLODEXTRIN:

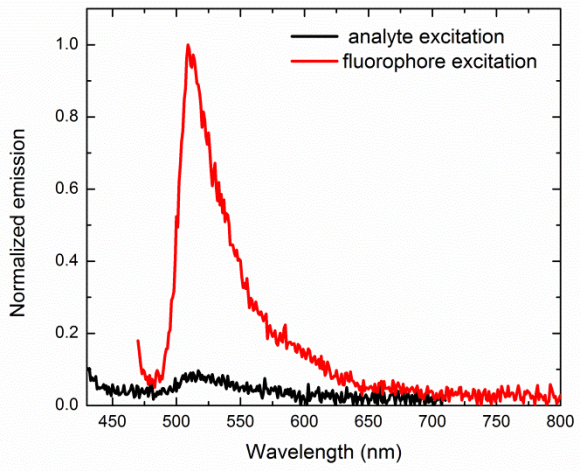
Analyte **1** – Fluorophore **8**



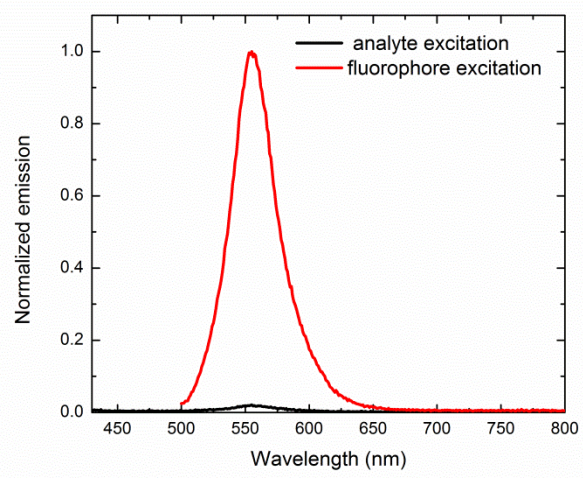
Analyte **1** – Fluorophore **10**



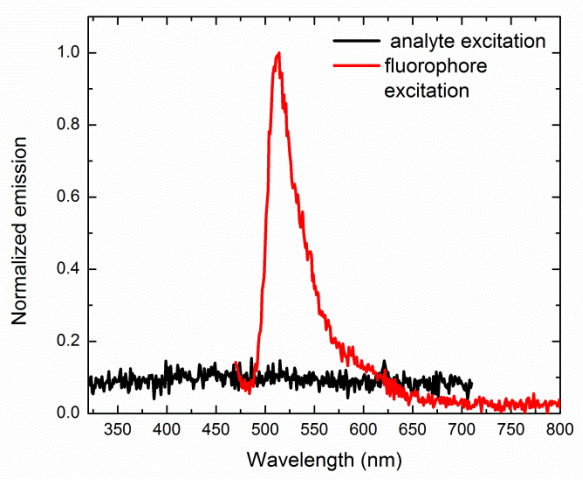
Analyte **2** – Fluorophore **8**



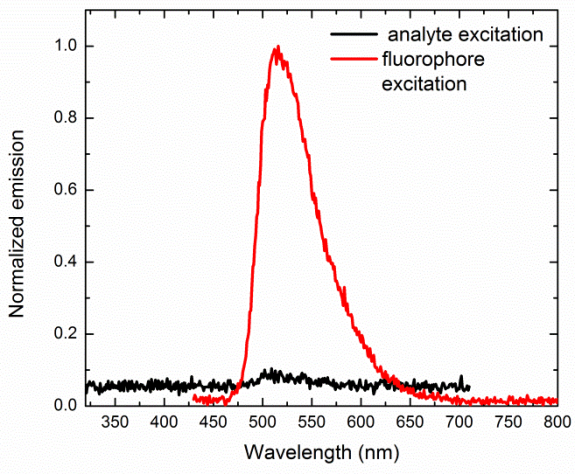
Analyte **2** – Fluorophore **10**



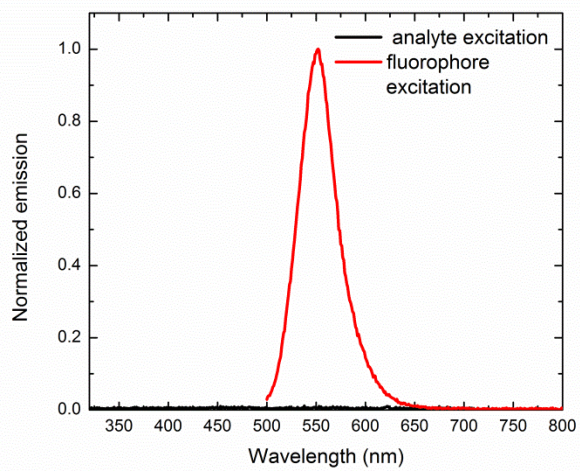
Analyte **3** – Fluorophore **8**



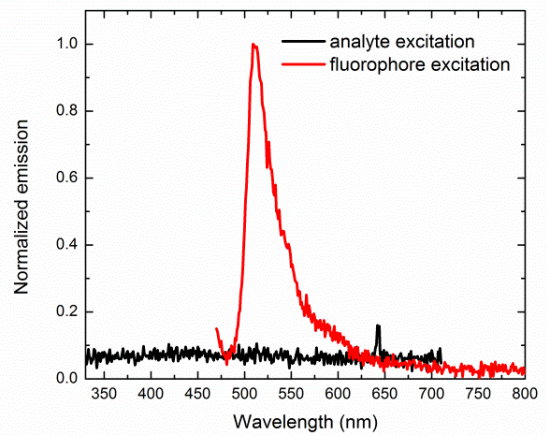
Analyte **3** – Fluorophore **9**



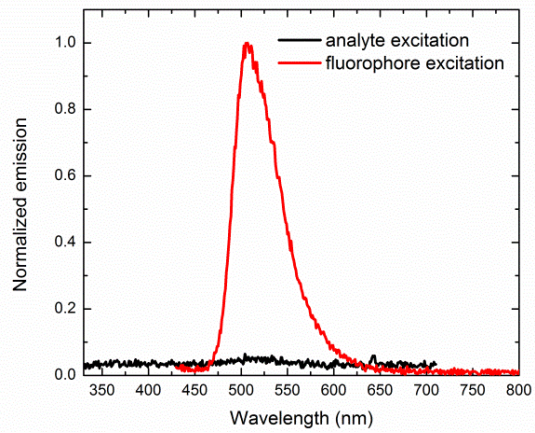
Analyte **3** – Fluorophore **10**



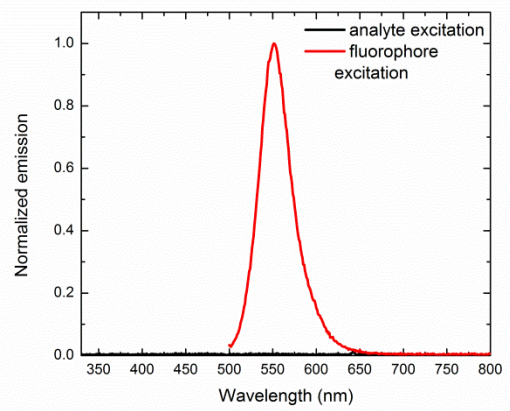
Analyte **4** – Fluorophore **8**



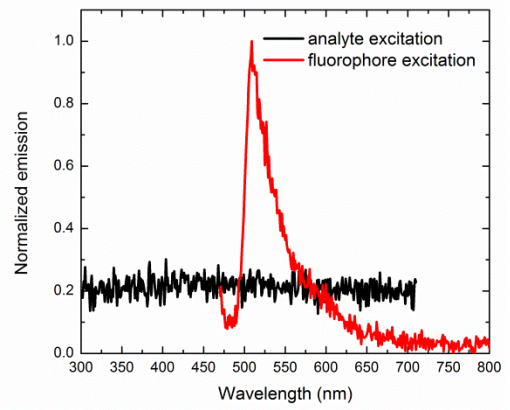
Analyte **4** – Fluorophore **9**



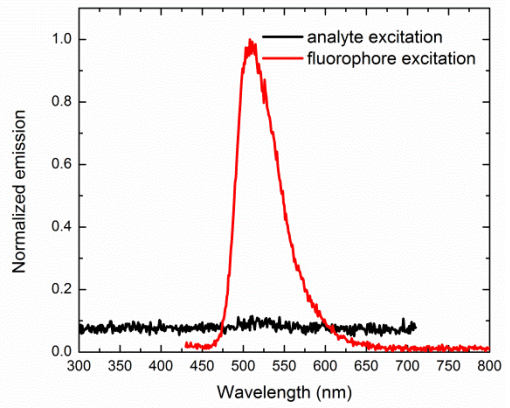
Analyte **4** – Fluorophore **10**



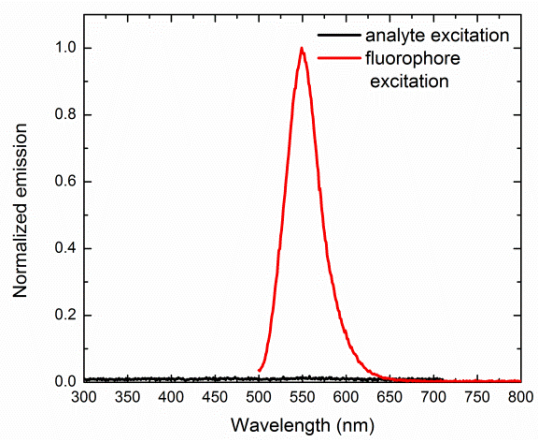
Analyte **5** – Fluorophore **8**



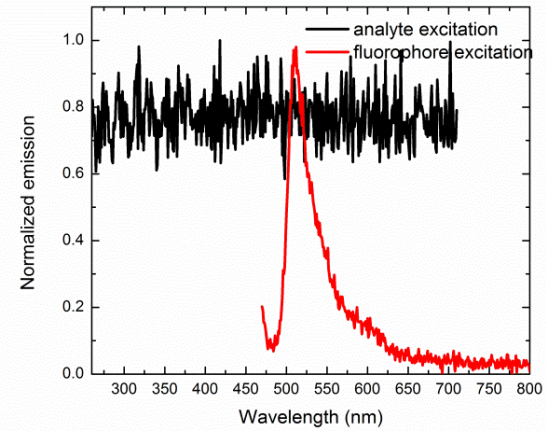
Analyte **5** – Fluorophore **9**



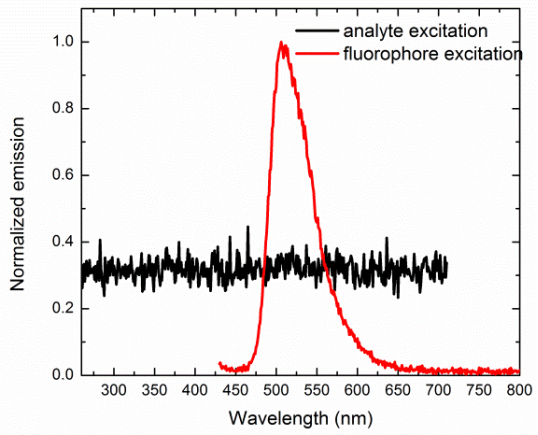
Analyte **5** – Fluorophore **10**



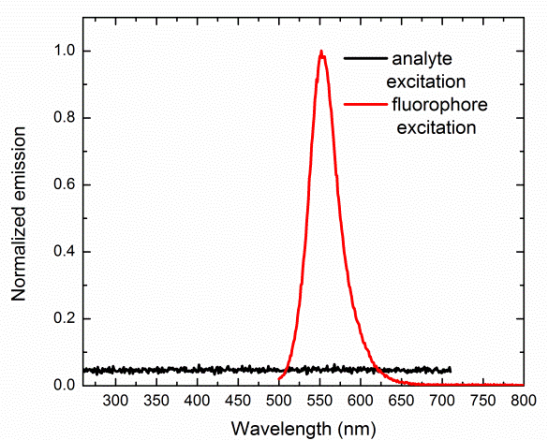
Analyte **6** – Fluorophore **8**



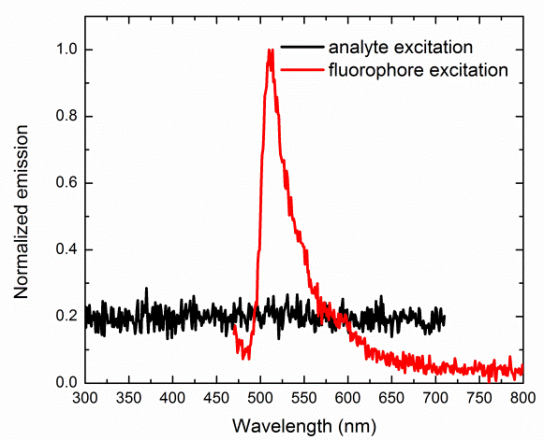
Analyte **6** – Fluorophore **9**



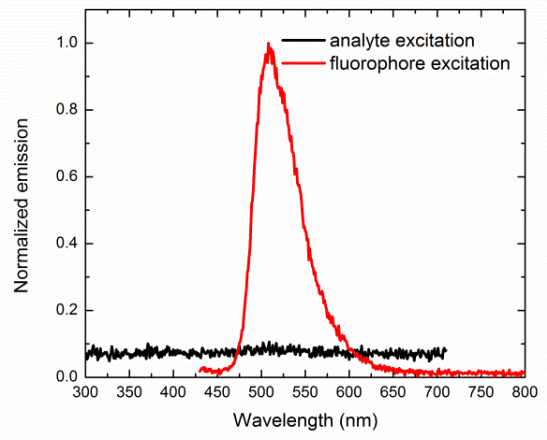
Analyte **6** – Fluorophore **10**



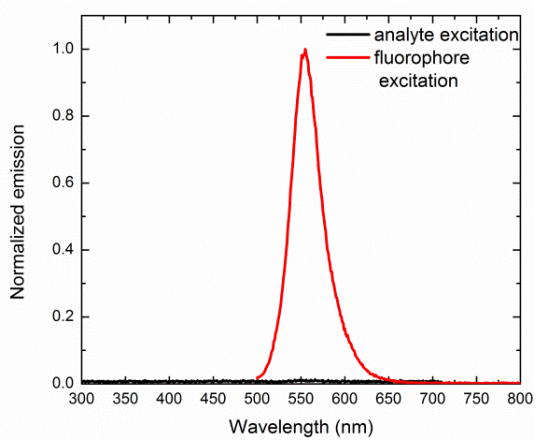
Analyte **7** – Fluorophore **8**



Analyte **7** – Fluorophore **9**



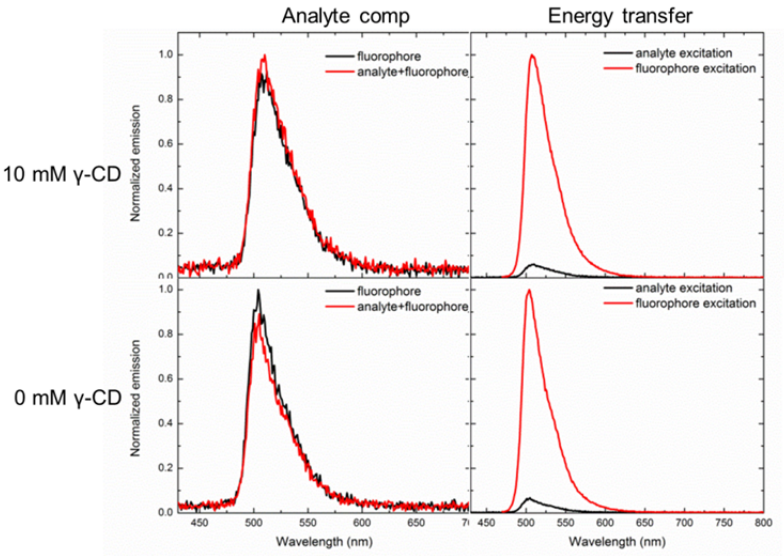
Analyte **7** – Fluorophore **10**



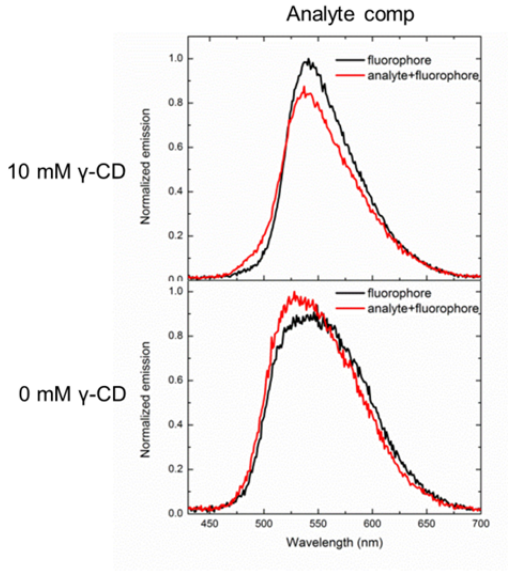
DOPED SOLUTIONS SUMMARY FIGURES:

IN PHOSPHATE BUFFERED SALINE:

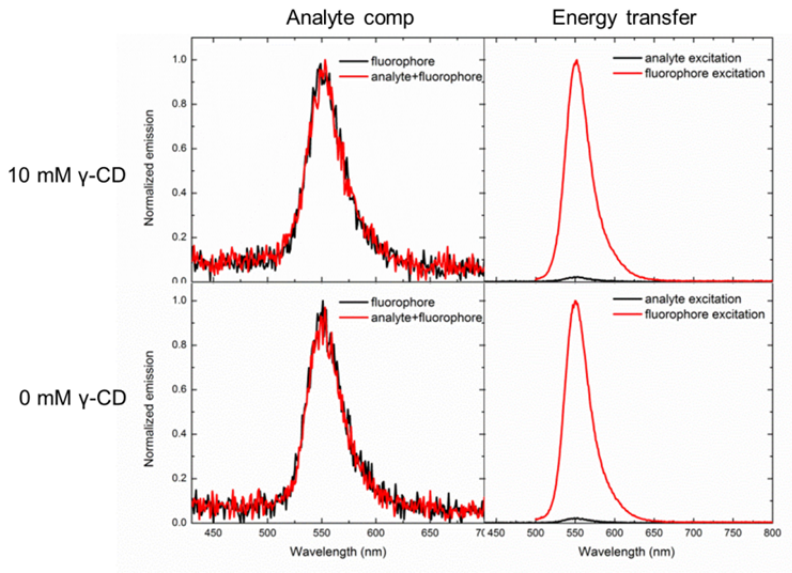
Analyte **1** – Fluorophore **8**



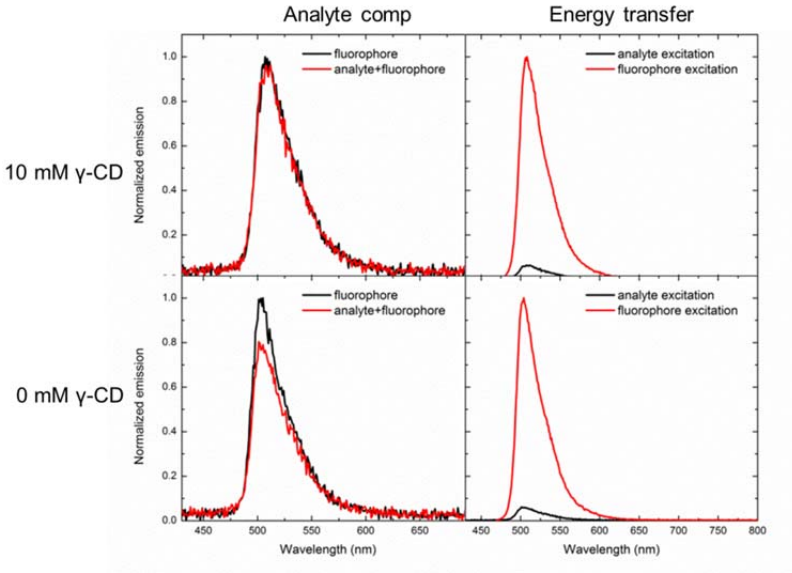
Analyte **1** – Fluorophore **9**



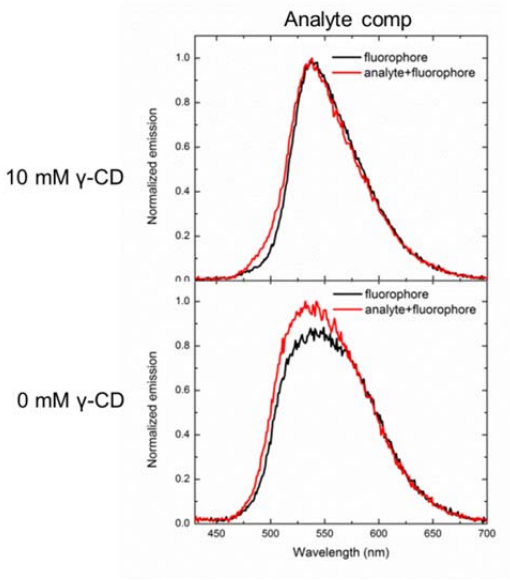
Analyte **1** – Fluorophore **10**



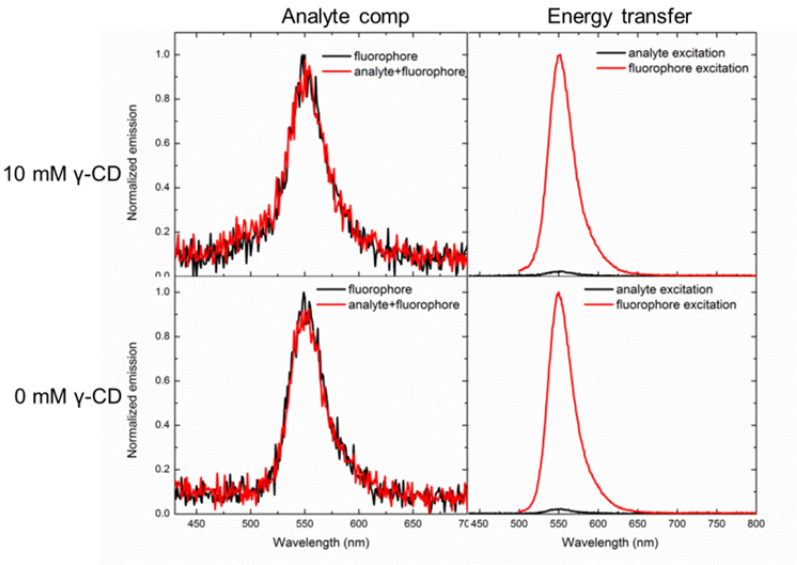
Analyte **2** – Fluorophore **8**



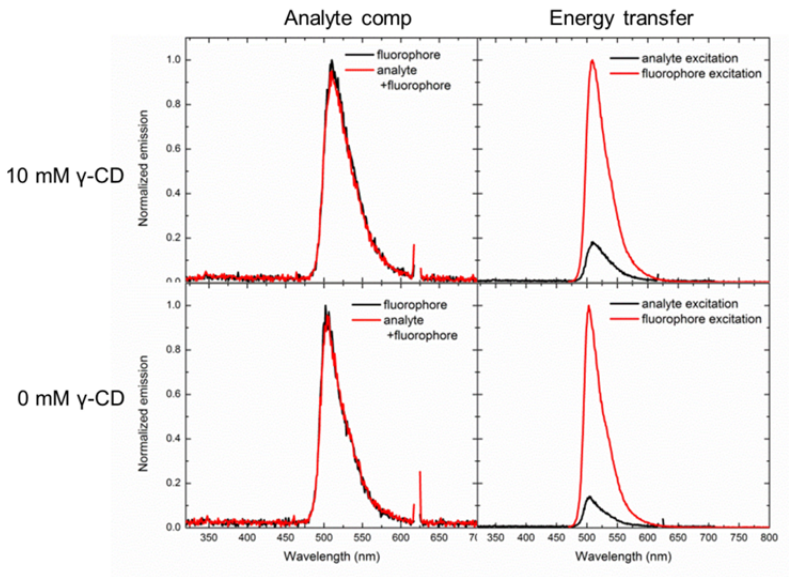
Analyte **2** – Fluorophore **9**



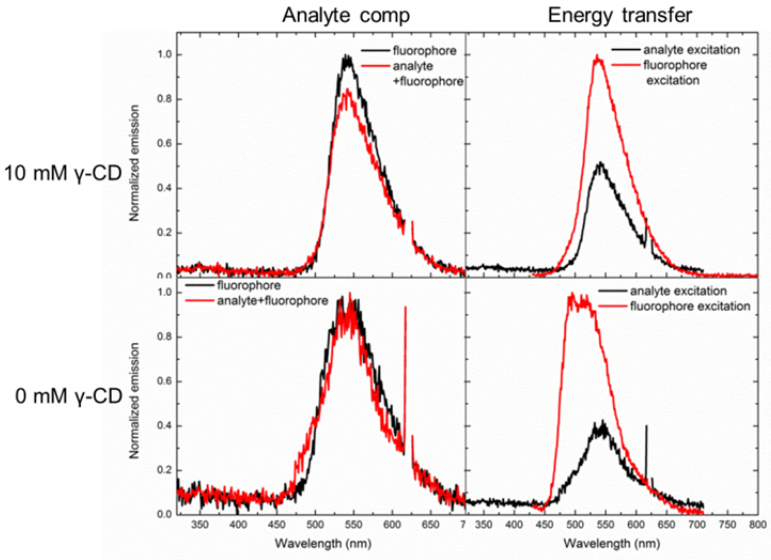
Analyte **2** – Fluorophore **10**



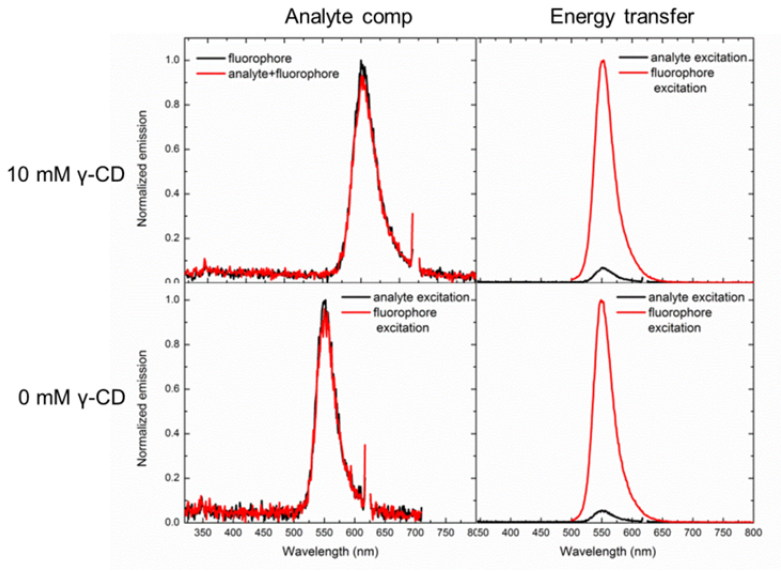
Analyte **3** – Fluorophore **8**



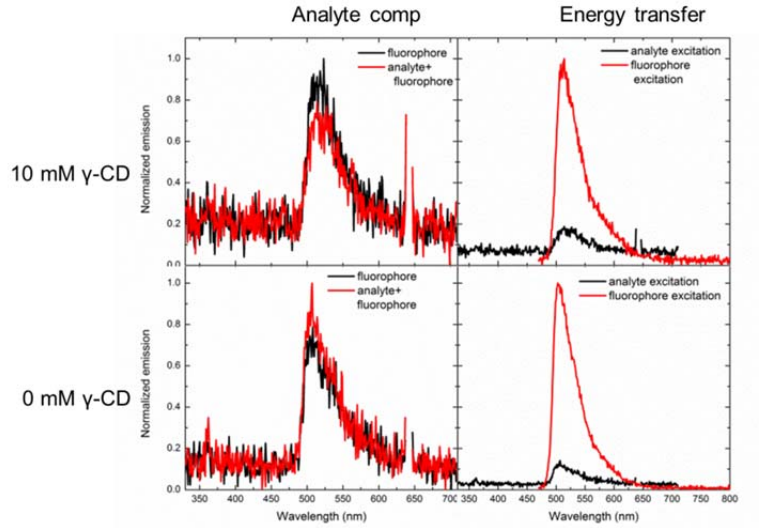
Analyte **3** – Fluorophore **9**



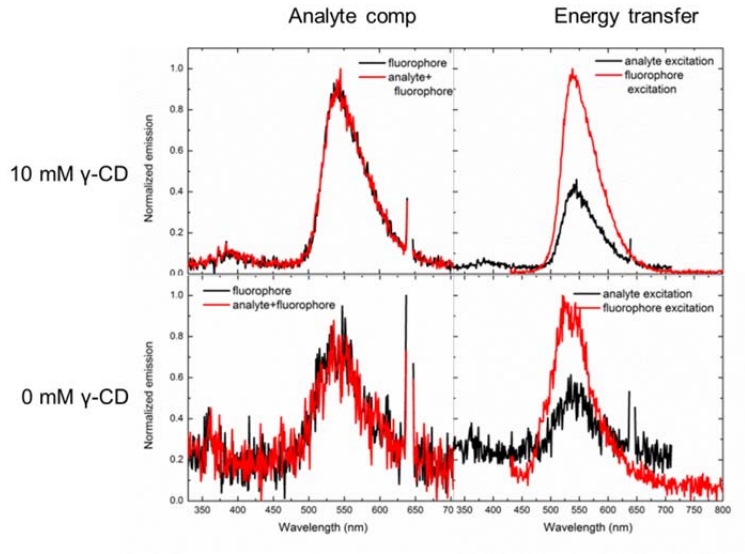
Analyte **3** – Fluorophore **10**



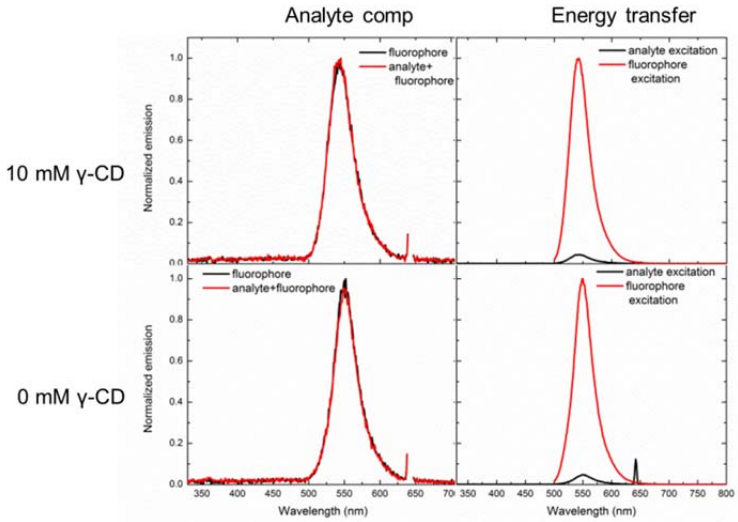
Analyte **4** – Fluorophore **8**



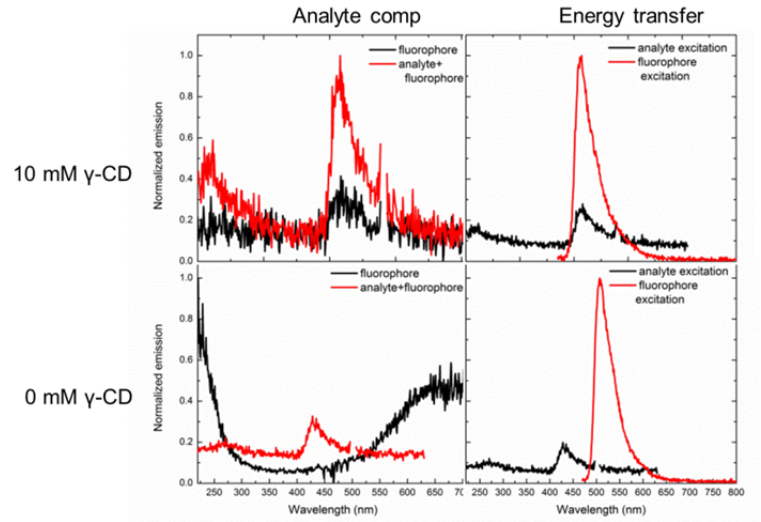
Analyte **4** – Fluorophore **9**



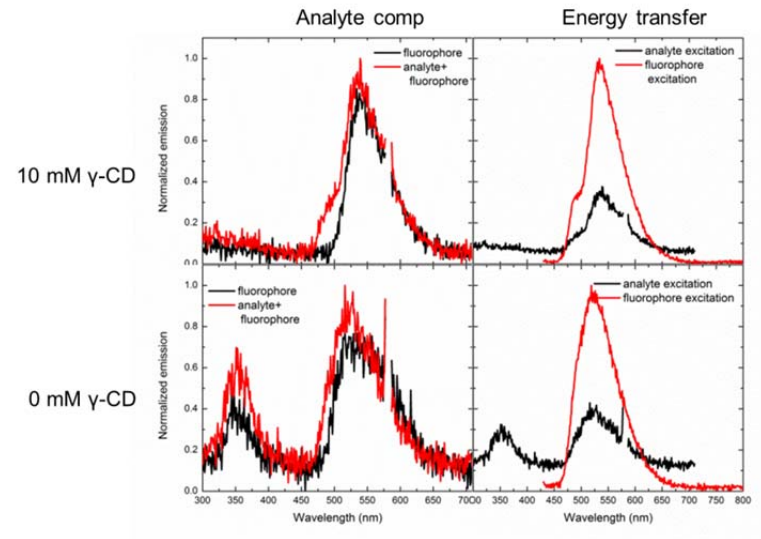
Analyte **4** – Fluorophore **10**



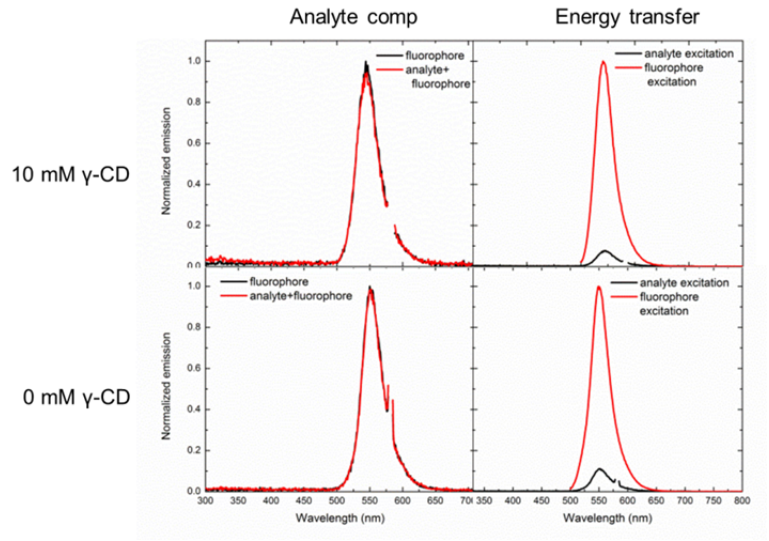
Analyte **5** – Fluorophore **8**



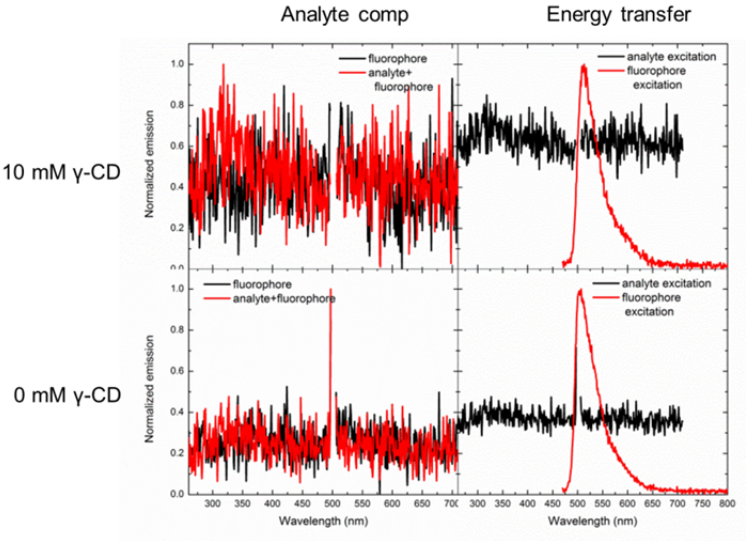
Analyte **5** – Fluorophore **9**



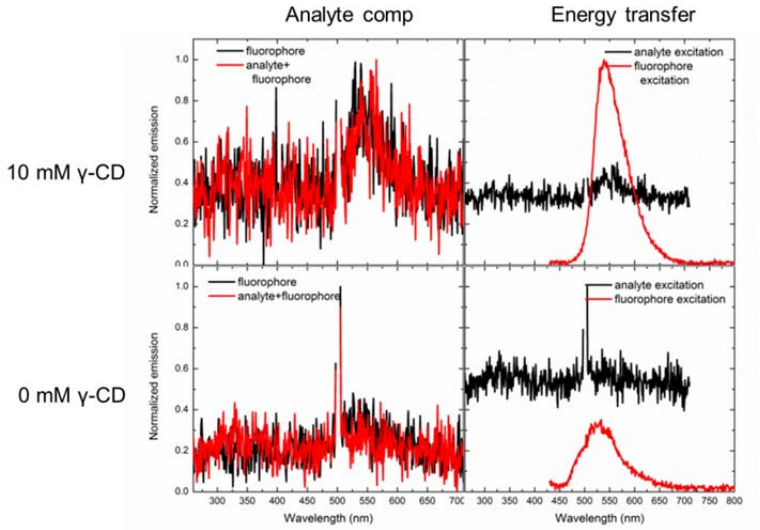
Analyte **5** – Fluorophore **10**



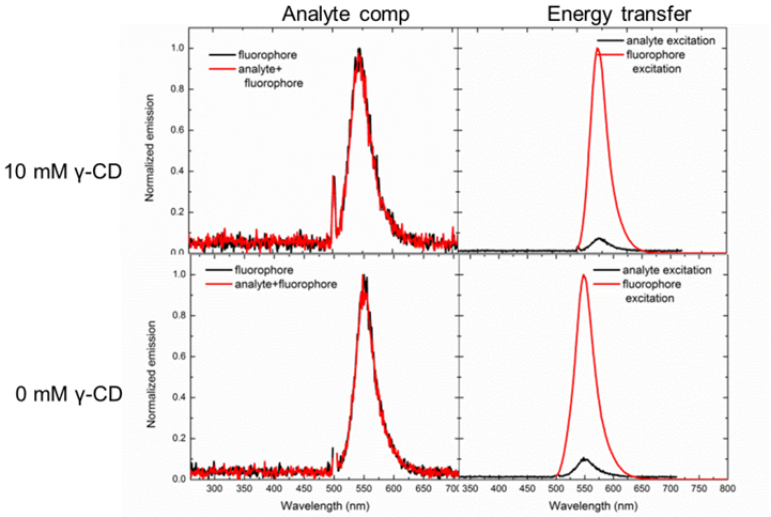
Analyte **6** – Fluorophore **8**



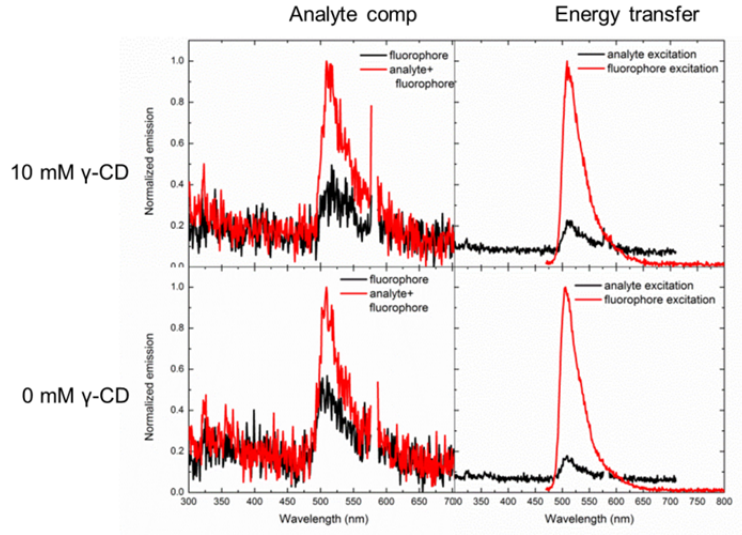
Analyte **6** – Fluorophore **9**



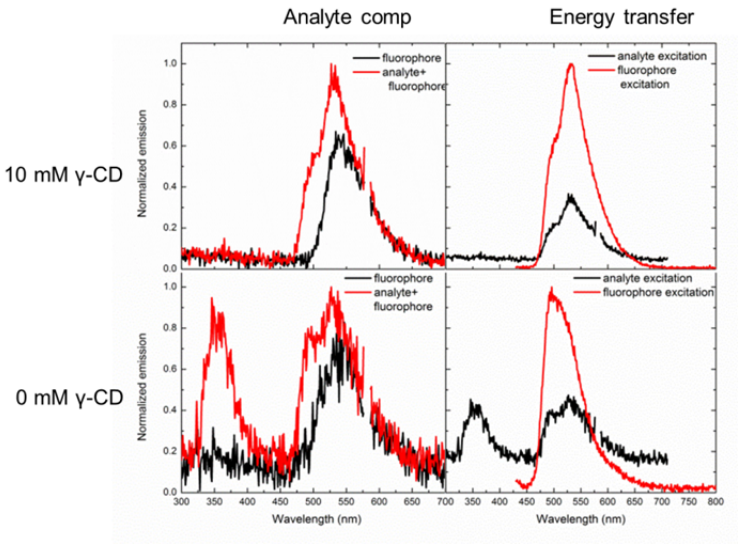
Analyte **6** – Fluorophore **10**



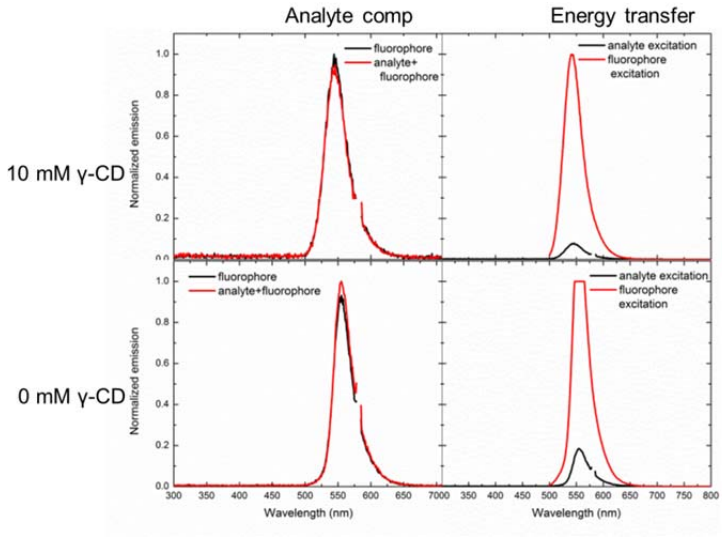
Analyte **7** – Fluorophore **8**



Analyte **7** – Fluorophore **9**

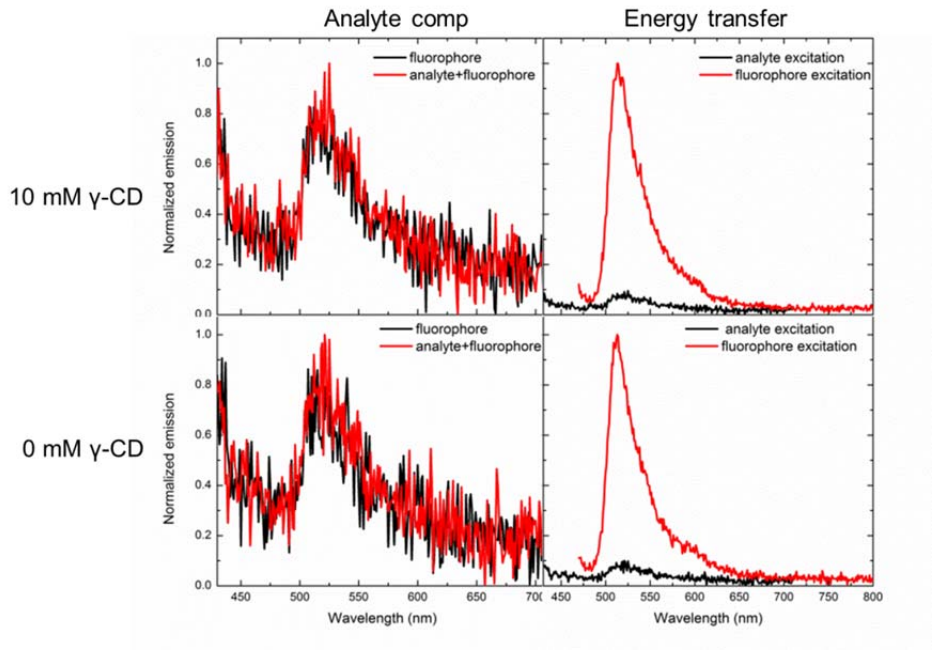


Analyte **7** – Fluorophore **10**

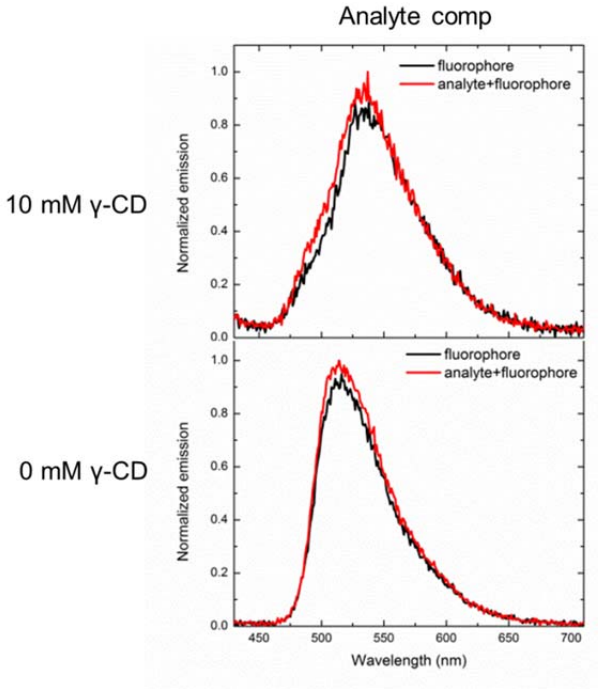


IN APPLE JUICE DOPED WITH γ-CYCLODEXTRIN AND ANALYTES:

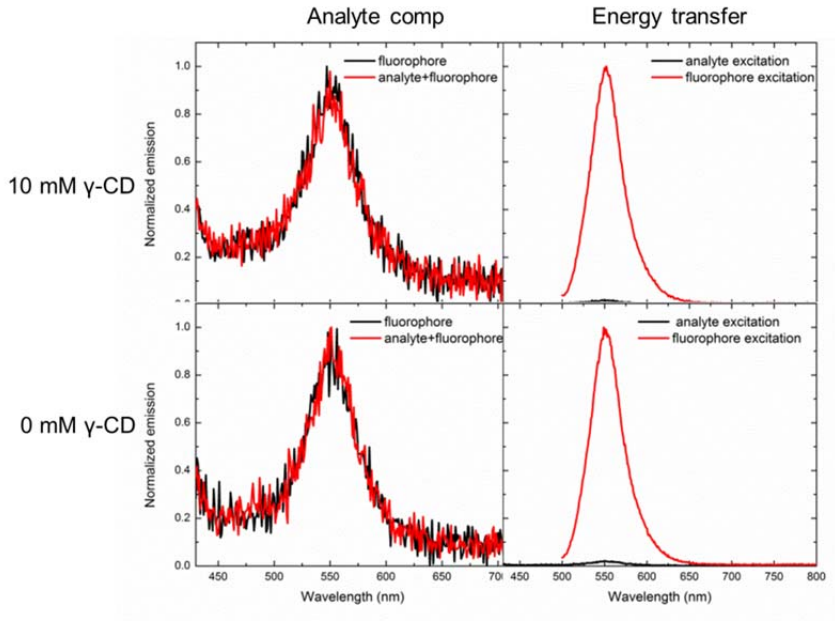
Analyte **1** – Fluorophore **8**



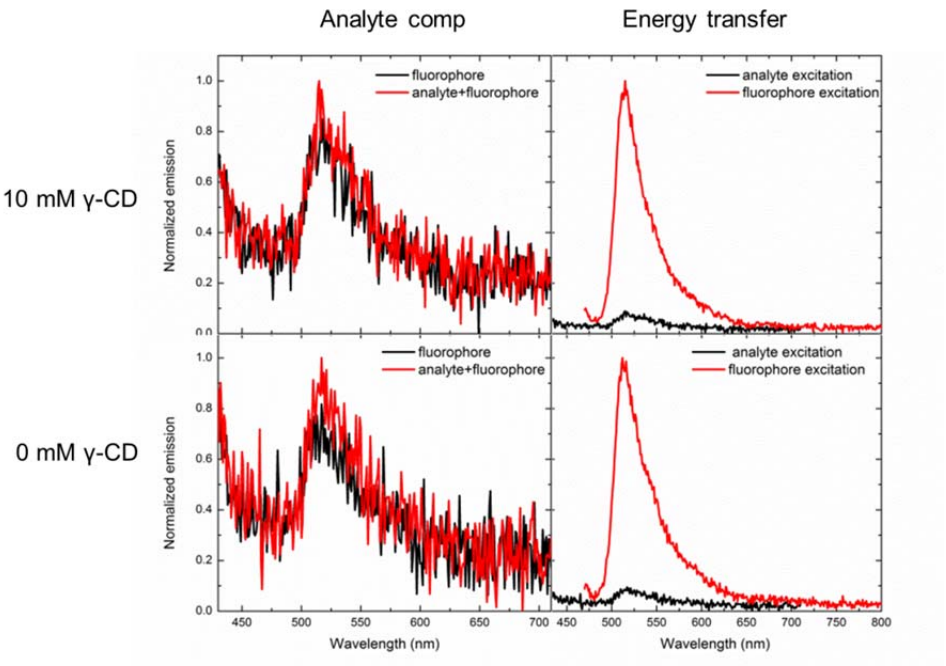
Analyte **1** – Fluorophore **9**



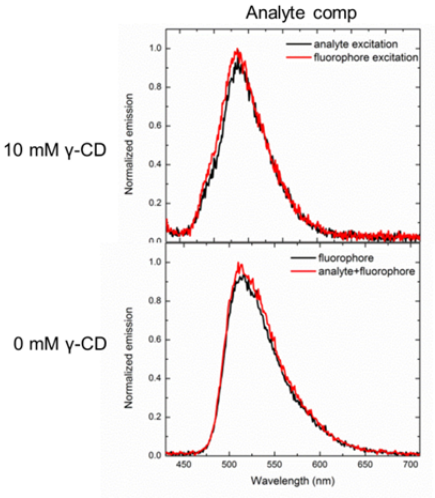
Analyte **1** – Fluorophore **10**



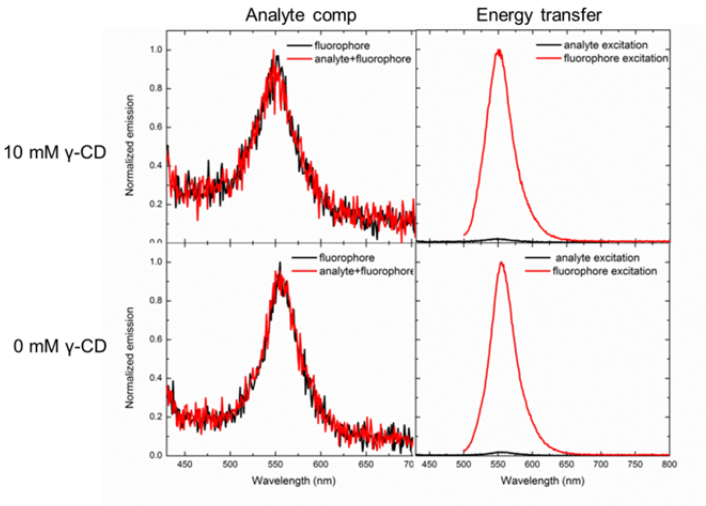
Analyte **2** – Fluorophore **8**



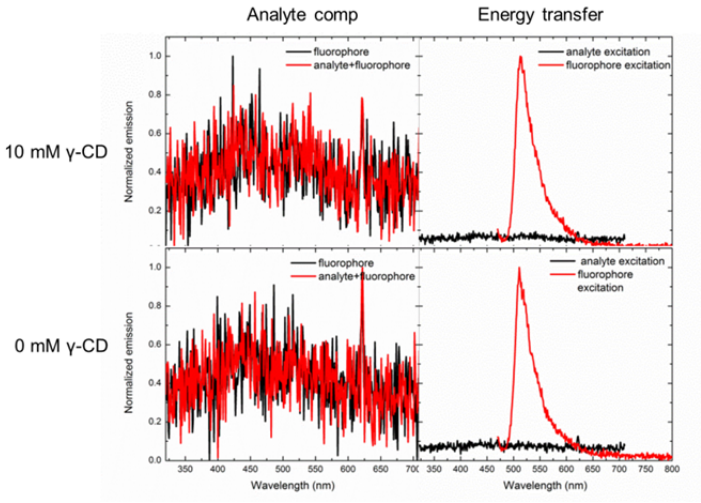
Analyte **2** – Fluorophore **9**



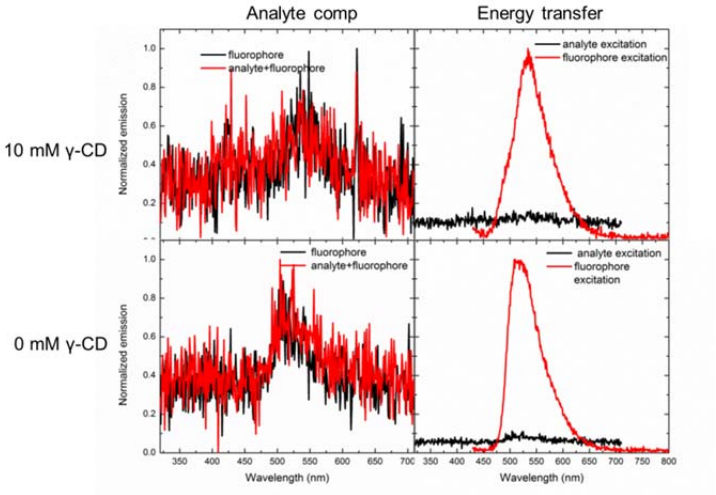
Analyte **2** – Fluorophore **10**



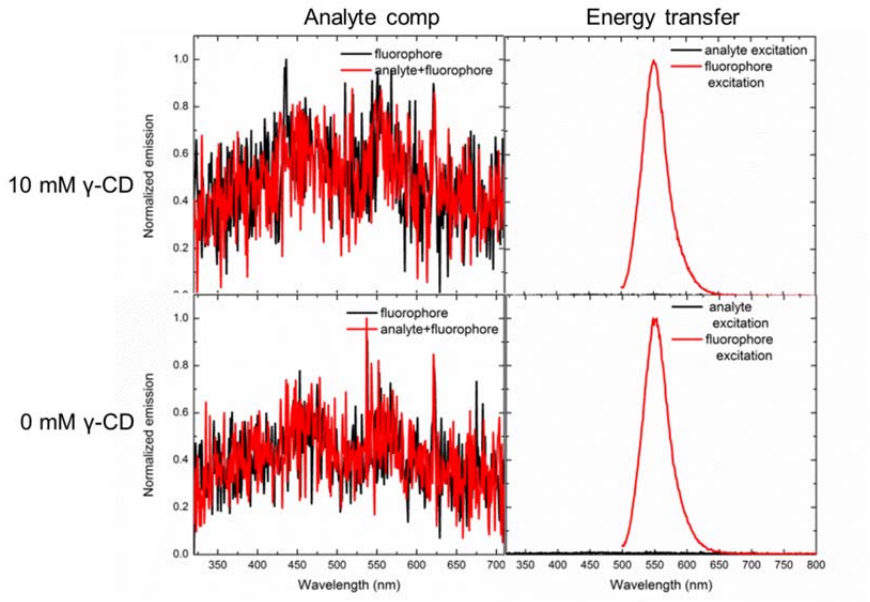
Analyte **3** – Fluorophore **8**



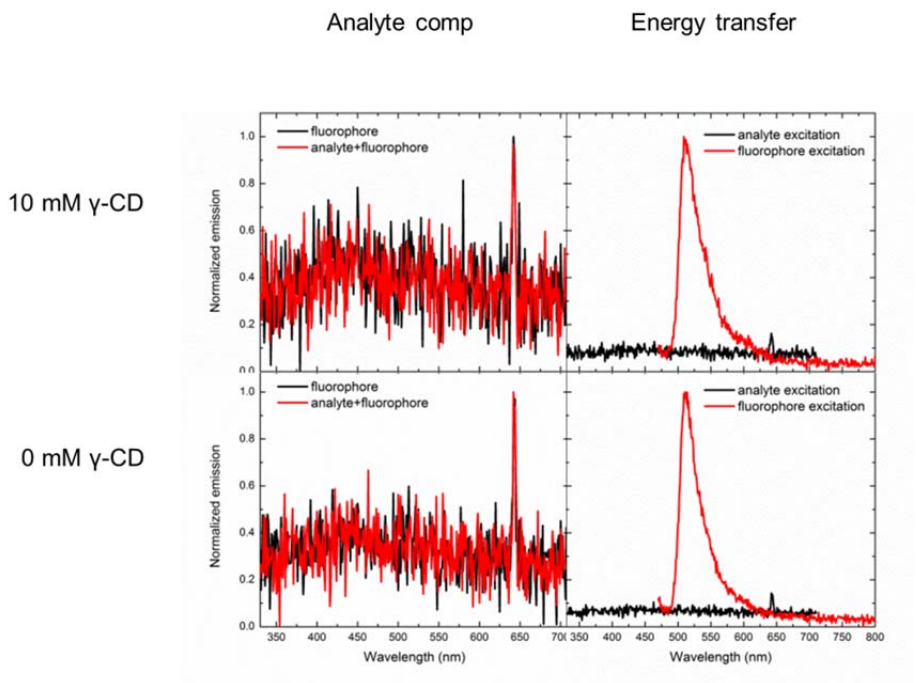
Analyte **3** – Fluorophore **9**



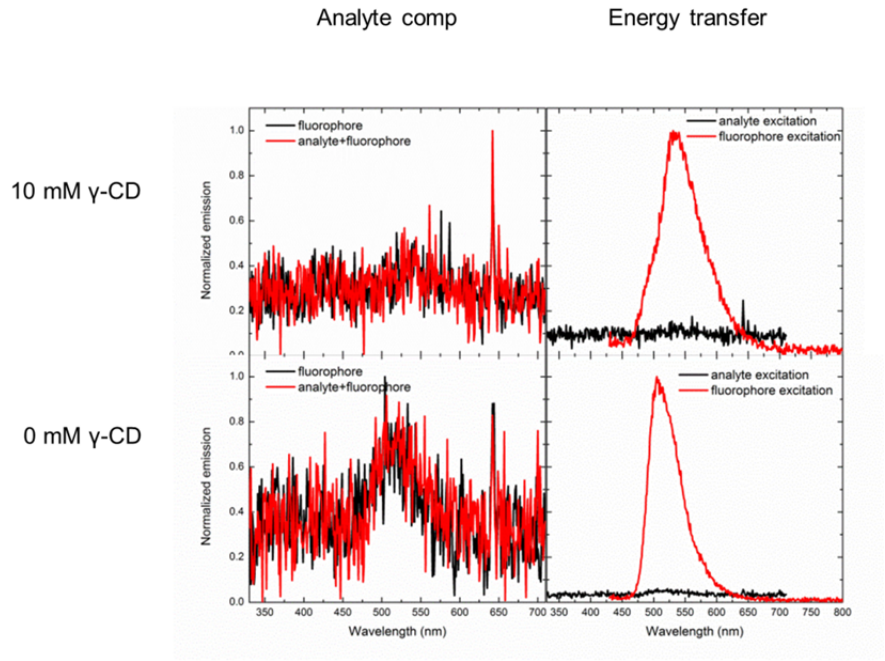
Analyte **3** – Fluorophore **10**



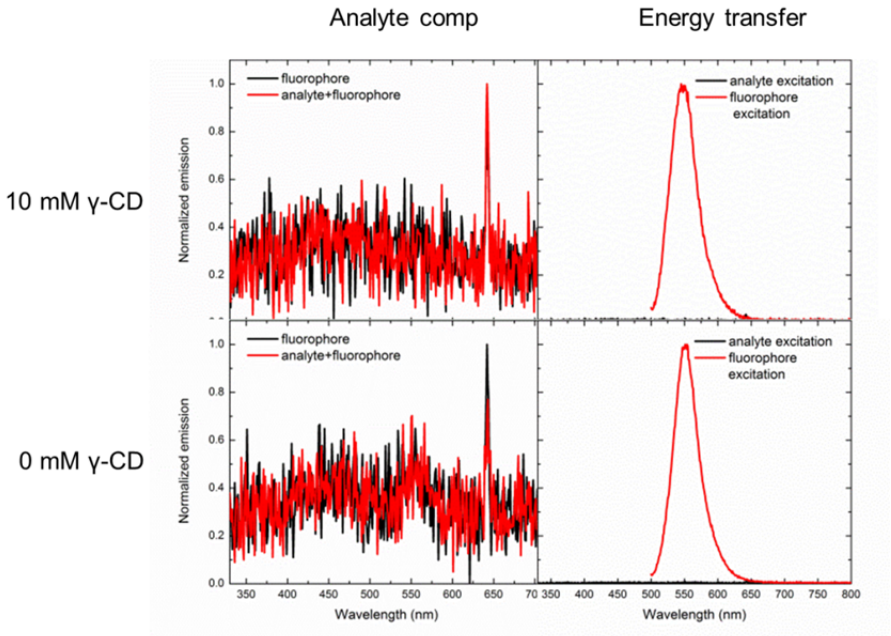
Analyte **4** – Fluorophore **8**



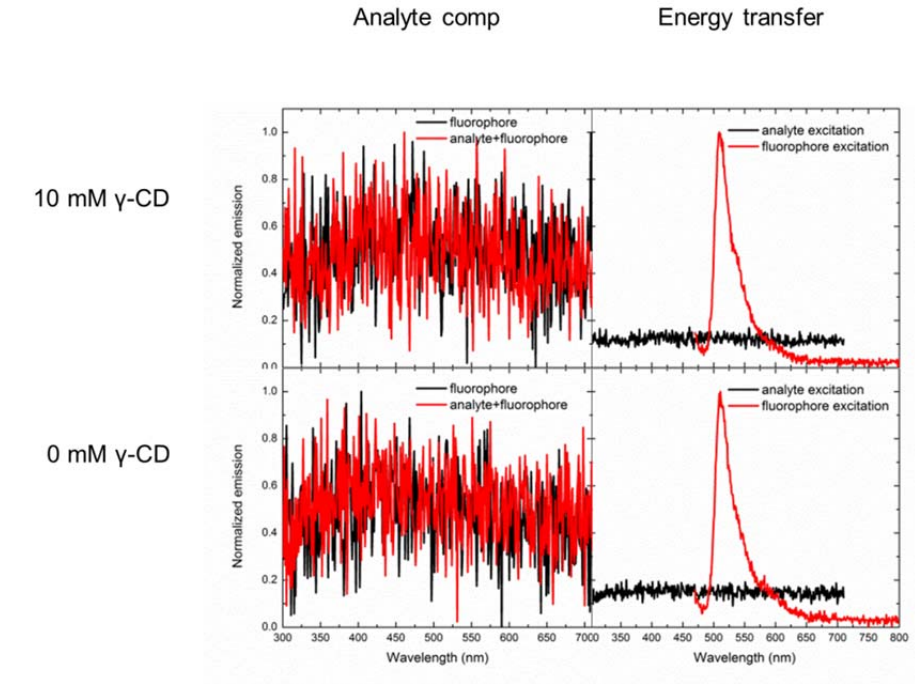
Analyte **4** – Fluorophore **9**



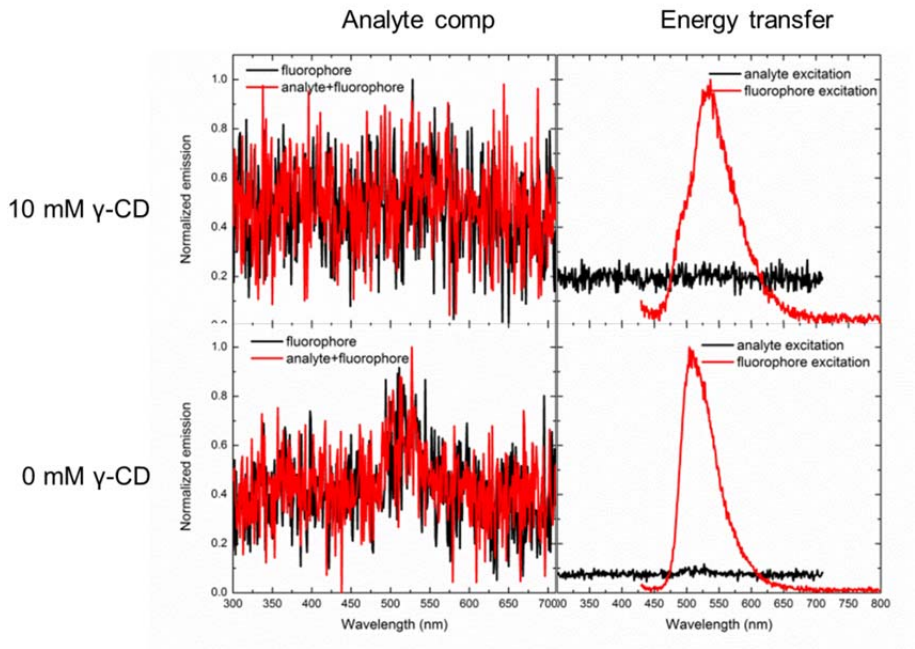
Analyte **4** – Fluorophore **10**



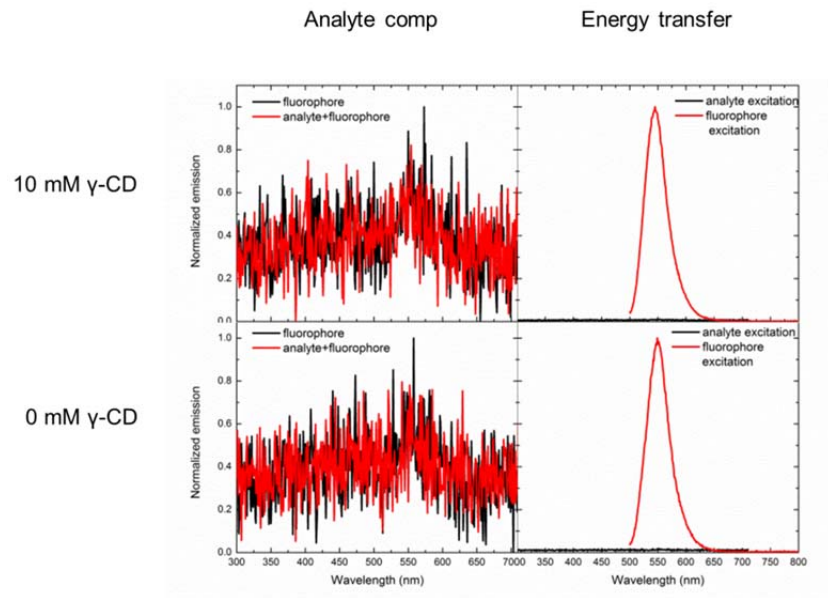
Analyte **5** – Fluorophore **8**



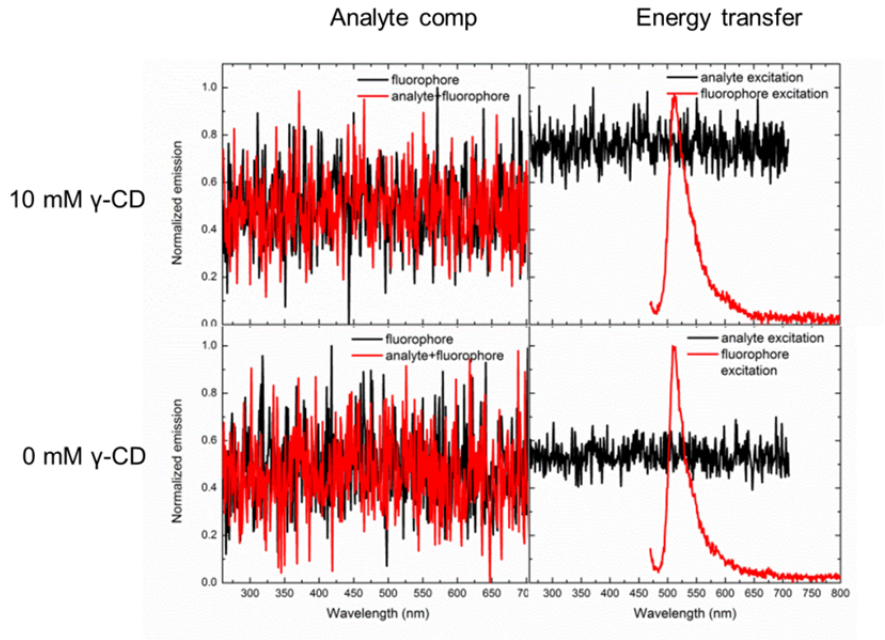
Analyte **5** – Fluorophore **9**



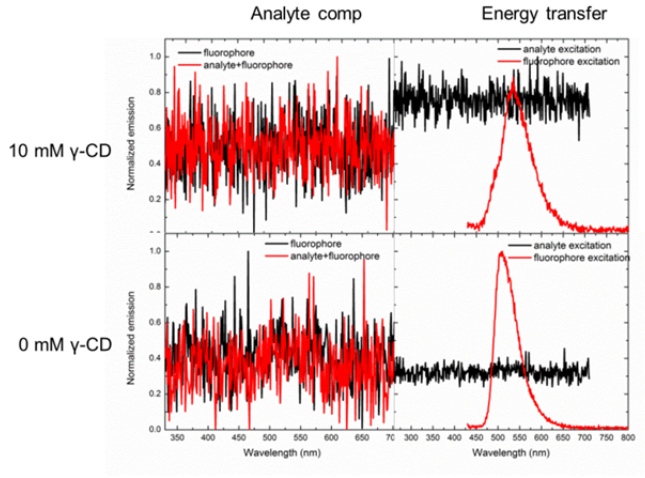
Analyte **5** – Fluorophore **10**



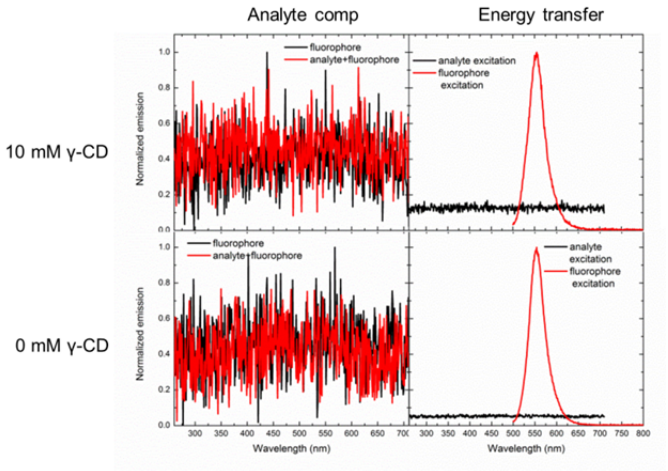
Analyte **6** – Fluorophore **8**



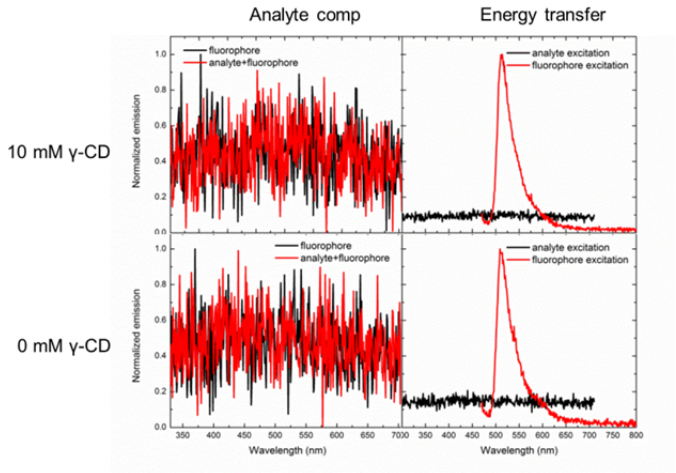
Analyte **6** – Fluorophore **9**



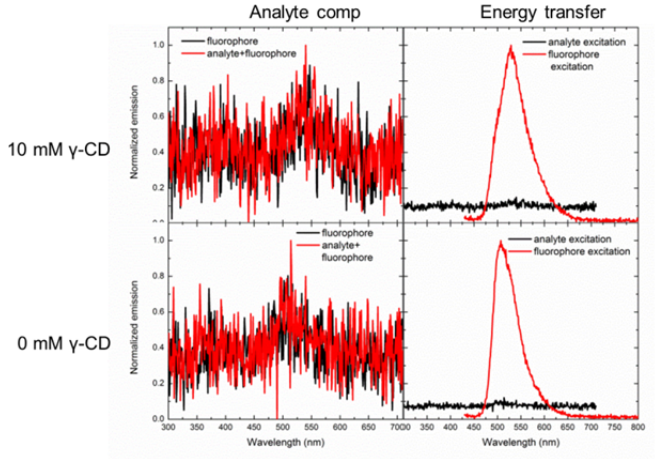
Analyte **6** – Fluorophore **10**



Analyte **7** – Fluorophore **8**



Analyte **7** – Fluorophore **9**



Analyte **7** – Fluorophore **10**