

Electronic Supporting Information for

**Turn-On Detection of Pesticides via Reversible Fluorescence
Enhancement of Conjugated Polymer Nanoparticles and Thin Films**

William Talbert, Daniel Jones, Joshua Morimoto, and Mindy Levine

TABLE OF CONTENTS

Materials and Methods.....	S3
Synthesis of Fluorescent Polymers.....	S4
Experimental Details.....	S5
Details for Limit of Detection Experiments.....	S6
Summary Tables	S7
Summary Figures of All Experimental Data.....	S8
Summary Figures for Dynamic Light Scattering Experiments.....	S8
Summary Figures for LOD Experiments.....	S10
Summary Figures for Absorbance Experiments.....	S11
Summary Figures for Fluorescence Experiments.....	S13
Summary Figures for Control Analytes.....	S17

MATERIALS AND METHODS

All the starting materials, reagents, and solvents were purchased from Sigma Aldrich, Acros Organics, TCI chemicals, Alfa Aesar, or Fisher Scientific and were used as received. All reactions were carried out under an inert atmosphere. Solvents were dried using an MBraun dual solvent purification system prior to use. Reactions were all monitored via analytical thin layer chromatography (TLC) using polyester backed TLC plates. Visualization was accomplished with UV light at 254 nm and/or with a KMnO_4 TLC stain. Product isolation was performed by using preparative TLC plates or silica gel chromatography. Both TLC plates and preparative TLC plates were purchased from Sorbent Technologies, GA. Column chromatography was performed with SiliaFlash F60 (230-400 mesh) silica gel, obtained from Silicycle Inc. Canada.

^1H NMR and ^{13}C NMR spectra were taken on a Bruker 300 MHz spectrometer and were recorded in CDCl_3 at ambient temperature. Fluorescence experiments were recorded on a Shimadzu RF 5301 spectrophotometer with 1.5 nm excitation and 3.0 nm emission slit widths for solution measurements and 1.5 nm excitation and 1.5 nm emission slit widths for thin films. Absorbance measurements were recorded on an Agilent 8453 UV-visible spectrophotometer at a concentration of 0.02 mg/mL.

Thin films were spin-cast onto 22 x 22 cm glass cover slips using a 1.0 mg/mL PFBO solution in chloroform at 1000 rpm for 20 seconds. For fluorescence experiments, slides were placed on top of a 20 mL vial containing iodine powder or a 1 mg/mL solution of DDT in THF for 10 seconds. The emission spectrum was recorded with the slides at a 45 degree angle relative to the beam.

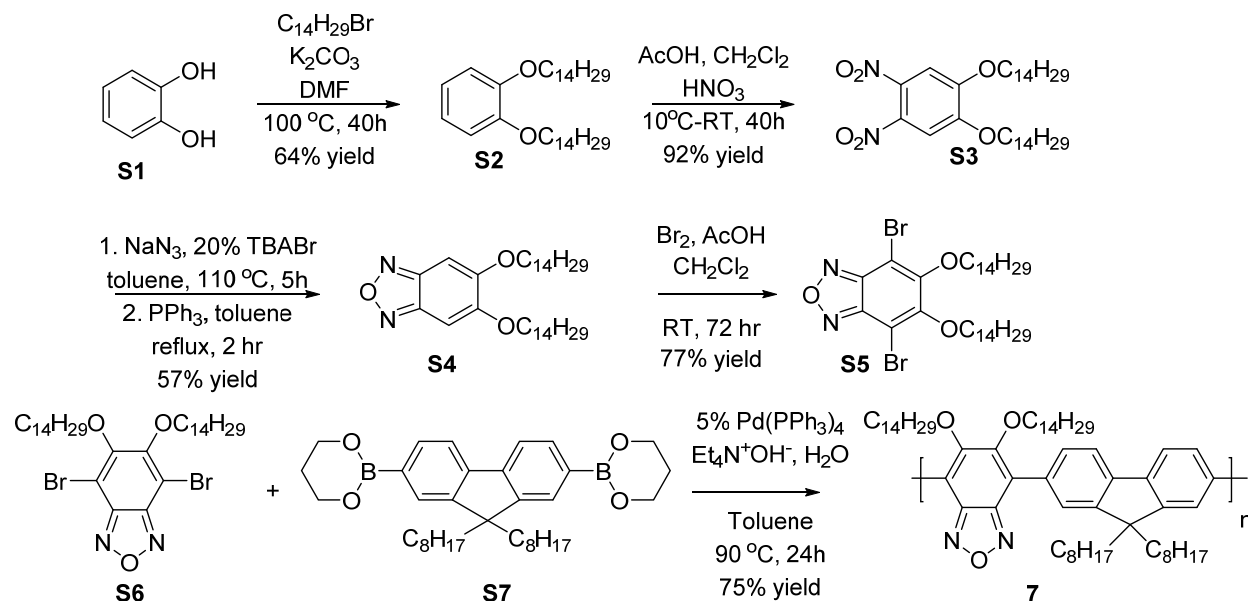
Dynamic light scattering experiments were run on a Malvern Zetasizer Nano ZS90, measuring particle size at 25°C and a 90° measurement angle, using Mark-Houwink parameters for the calculation of molecular weight.

Gel permeation chromatography (GPC) data were obtained using an Agilent Infinity GPC system equipped with three Agilent PL Gel columns 7.5mm x 300mm (5 μm , pore sizes: 103, 104 and 105 Å). Molecular weight and M_w/M_n ratios were determined versus PS standards (500g/mol – 3150kg/mol; obtained from Polymer Laboratories).

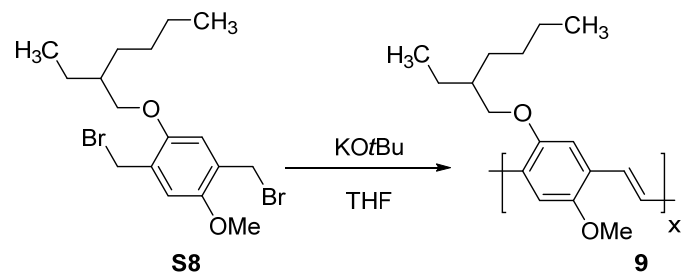
SYNTHESIS OF FLUORESCENT POLYMERS

Fluorescent polymer **7** was synthesized following procedures described in the references below. All chemical intermediates and products were fully characterized using ^1H and ^{13}C NMR spectroscopy.

References: Helgesen, M.; Gevorgyan, S. A.; Krebs, F. C.; Janssen, R. E. J. "Substituted 2,1,3-Benzothiadiazole- and Thiophene-Based Polymers for Solar Cells – Introducing a New Thermocleavable Precursor." *Chem. Mater.* **2009**, *21*, 4669-4675; Bouffard, J.; Swager, T. M. "Fluorescent Conjugated Polymers that Incorporate Substituted 2,1,3-Benzoxadiazole and 2,1,3-Benzothiadiazole Units." *Macromolecules* **2008**, *41*, 5559-5562.



Fluorescent polymer **9** was synthesized following procedures described in the reference below. All chemical intermediates and products were fully characterized using ^1H and ^{13}C NMR spectroscopy.



Synthesis: 200 mg of 2,5-bis(bromomethyl)-1-methoxy-4-(2-ethylhexyloxy)benzene **S8** (0.47 mmol, 1.0 equivalents) was dissolved in 5.0 mL of oxygen- and moisture-free tetrahydrofuran (THF), and cooled to 0 °C. 210 mg of potassium tert-butoxide (1.9 mmol, 4.0 equivalents) were dissolved in 2.0 mL of anhydrous THF and added via syringe to the solution of **1** in THF. After 2 hours of vigorous stirring at 0 °C, the reaction mixture was quenched via the addition of a five-fold volume of methanol (approximately 35 mL), and polymer **2** was isolated by vacuum filtration. The polymer was dissolved in deuterated chloroform for ^1H NMR analysis.

References: Mako, T.; Levine, M. "Synthesis of a Fluorescent Conjugated Polymer in the Undergraduate Organic Teaching Laboratory." *J. Chem. Educ.* **2013**, *90*, 1376-1379.

EXPERIMENTAL DETAILS

DETAILS OF NANOPARTICLE FABRICATION

PFBO nanoparticles were formed following a modified literature-reported procedure. 2 mL of polymer solution (2 mg/mL) in THF was added to 8 mL of deionized sonicating water. The solution was allowed to sonicate for 30 minutes, at which point the THF was removed by bubbling nitrogen through the solution for 1 hour. An additional 2 mL of deionized water was added to the solution to make a 0.2 mg/mL stock nanoparticle solution.

DETAILS OF THIN FILM FABRICATION

Thin films were spin-cast onto 22 x 22 cm glass cover slips using a 1 mg/mL PFBO solution in chloroform at 1000 rpm for 20 seconds. For fluorescence experiments, slides were placed on top of a 20 mL vial containing iodine powder or a 1 mg/mL solution of DDT for 10 seconds. The emission spectrum was recorded after 10 seconds had passed. The thin film was mounted at a 45 degree angle relative to the beam.

DETAILS FOR RELATIVE QUANTUM YIELD DETERMINATION

To determine quantum yield, 5-6 solutions of each nanoparticle solution were made, with absorbances ranging from 0.01 to 0.1 (arbitrary absorption units). The fluorescence emission of each solution was recorded. The integrated fluorescence signal was then plotted against the absorbances and a trendline was determined. The quantum yield (Q) was determined through comparison to standards using the equation:

$$Q = Q_R * (M/M_R) * (n^2/n_R^2);$$

where M is the slope of the absorbance verse fluorescence trace and n is the refractive index of the media.

Rhodamine B, Rhodamine 6G, and Fluorescein were the standards used to determine quantum yield. The solutions were excited at the following wavelengths: Rhodamine B: 545 nm; Rhodamine 6G: 530 nm.

FLUORESCENCE EXPERIMENTAL DETAILS

For fluorescence experiments, two solutions were prepared: one containing dilute PFBO nanoparticles in water (Solution A), and one containing dilute pesticide (**1-10**) in acetonitrile (Solution B). For each run, 2 mL of solution A (1.25 E-3 mg/mL) were added to the cuvette and mixed with 0.5 mL of solution B (0 – 70 μ M small molecule). The optimal concentration for these solution-state fluorescence experiments was determined through testing a variety of polymer concentrations and looking for the one that gave reproducible data over several trials and with several different polymer-pesticide combinations. The polymers were excited at the following wavelengths: polymer **7**: 420 nm; polymer **8**: 490 nm; polymer **9**: 500 nm; polymer **10**: 480 nm; polymer **11**: 480 nm.

DYNAMIC LIGHT SCATTERING DETAILS

To study the size of the nanoparticles, dynamic light scattering (DLS) was used. DLS data were obtained using a Malvern Zetasizer Nano S. A 0.0125 mg/mL solution of PFBO nanoparticles in H₂O was used to determine the Z-average (particle diameter) and polydispersity indices (PDI) of the nanoparticles.

DETAILS FOR LIMIT OF DETECTION EXPERIMENTS

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

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

To determine the limit of detection (LOD) and limit of quantification (LOQ), each fluorophore-analyte combination was examined in the following manner: 2 mL of PFBO nanoparticles in H₂O (1.25 e⁻³ mg/mL) was added to a cuvette, then 100 μL of analyte solution (1 mg/mL) in acetonitrile was added in 20 μL portions. All solutions were excited at 420 nm, and fluorescence emission spectra were recorded 6 times for each addition of analyte.

All fluorescence emission spectra were integrated versus wavenumber. Calibration curves were created with analyte concentration (in μM) on the X-axis and the integrated fluorophore emission of the Y-axis. The curve was fitted with a trend line and a corresponding equation for the line was determined.

For the LOD, the limit of the blank was defined by 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.

The LOB value was then inserted into the line equation as the Y-value, and the X-value was solved for, giving the LOD in μM.

For the LOQ, the limit of the blank was defined by the following equation:

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

The LOB value was then inserted into the line equation as the Y-value, and the X-value was solved for, giving the LOQ in μM.

SUMMARY TABLES

SUMMARY TABLES FOR THIN FILM EXPERIMENTS

Ratio of fluorescence in thin films with DDT and I₂ additions:

Ratio is defined as the integrated fluorescence of the film under a given set of experimental conditions to the integrated fluorescence of the film before treatment with any analyte or reagent.

	ratio
blank	1.00
with DDT	1.27
with I ₂	1.06

	ratio
blank	1.00
with I ₂	0.91
with DDT	1.04

SUMMARY TABLE FOR LOD EXPERIMENTS:

Analyte	Equation	R ²	LOD (μM)
1	y = 210.09x + 38261	0.9777	4.6
2	y = 1929.2x + 453988	0.8212	83.1
3	y = 1895.3x + 434915	0.9463	69.3
4	y = 2454.3x + 292818	0.9789	58.2

SUMMARY TABLES FOR CONTROL ANALYTE EXPERIMENTS

Fluorescence Modulation with 70 μM of Analyte

Analyte	Modulation
Bisphenol A (BPA)	0.68 ± 0.01
<i>o</i> -dichlorobenzene	0.68 ± 0.01
Diphenylmethane	0.75 ± 0.01
1,1-Diphenylpropane	0.77 ± 0.00
<i>m</i> -Xylene	0.63 ± 0.00
<i>o</i> -Xylene	0.65 ± 0.01
<i>p</i> -Xylene	0.63 ± 0.00

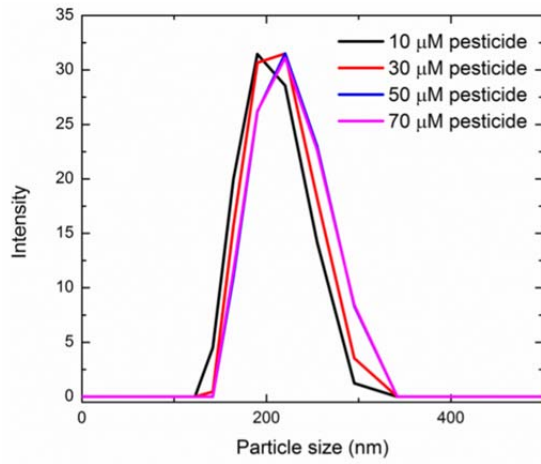
SUMMARY TABLE FOR QUANTUM YIELD EXPERIMENTS

Quantum yield for polymer nanoparticles

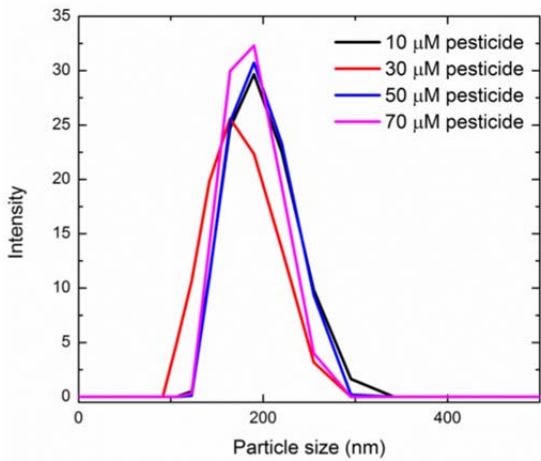
Fluorophore	Lit Value ¹	Calculated Values
Rhodamine 6G	0.95	0.98
Rhodamine B	0.50	0.56
Fluorescein	0.95	0.93
Polymer 8	-	0.69
Polymer 9	-	0.34
Polymer 10	-	0.25
Polymer 11	-	0.91

SUMMARY FIGURES OF ALL EXPERIMENTAL DATA
SUMMARY FIGURES FOR DYNAMIC LIGHT SCATTERING EXPERIMENTS

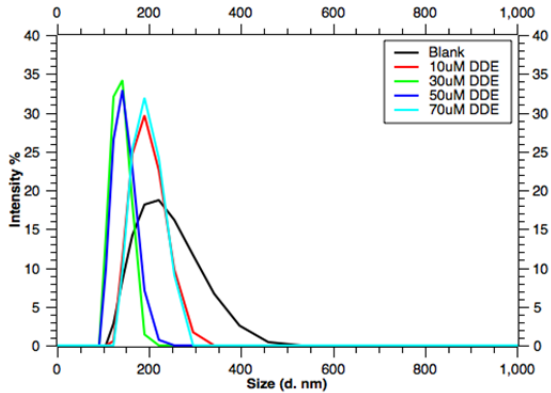
Analyte 1



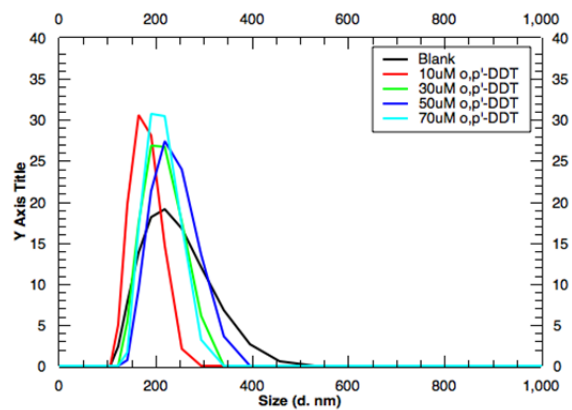
Analyte 2



Analyte 3

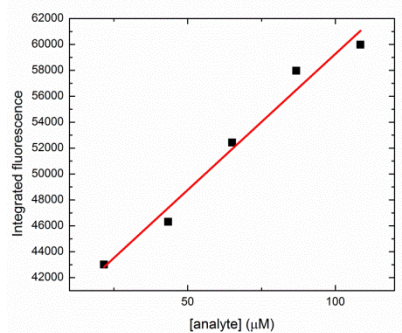


Analyte 4

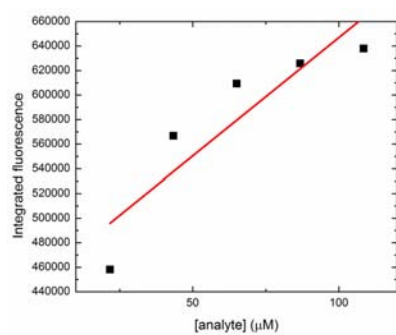


SUMMARY FIGURES FOR LIMIT OF DETECTION EXPERIMENTS

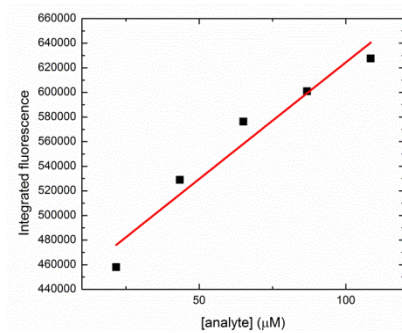
Analyte 1:



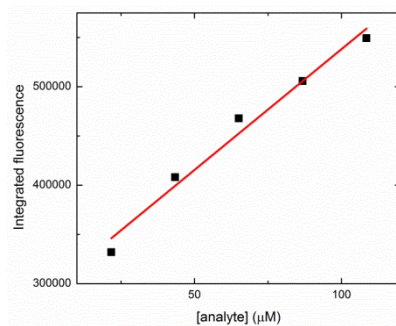
Analyte 2:



Analyte 3:

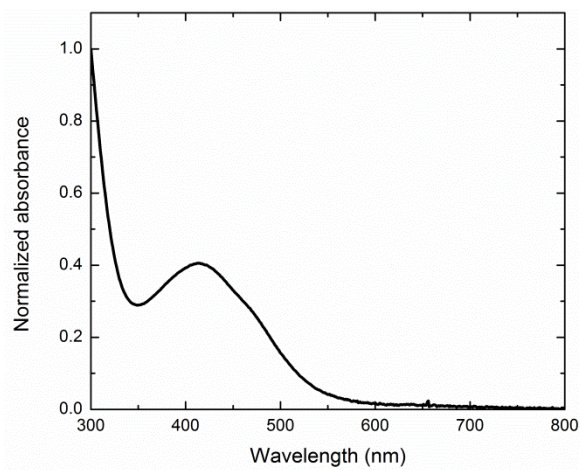


Analyte 4:

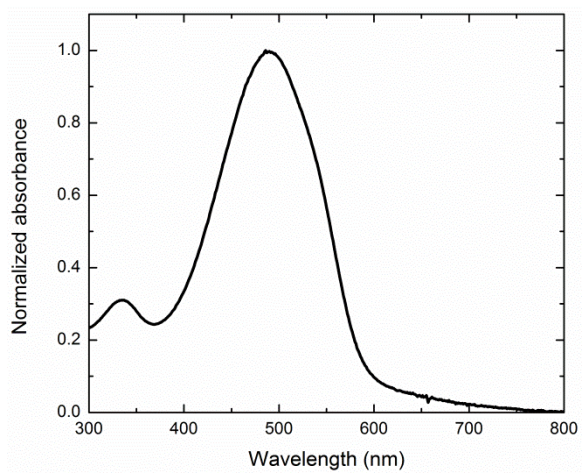


SUMMARY FIGURES FOR ABSORBANCE EXPERIMENTS

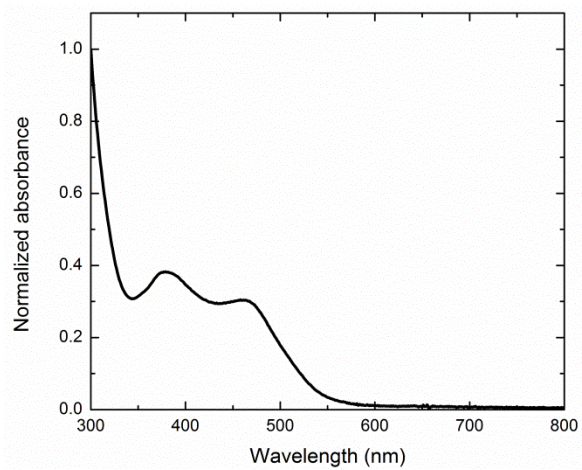
Polymer 8



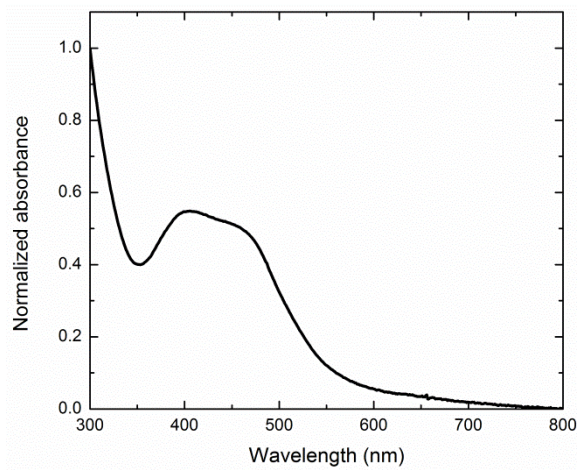
Polymer 9



Polymer 10



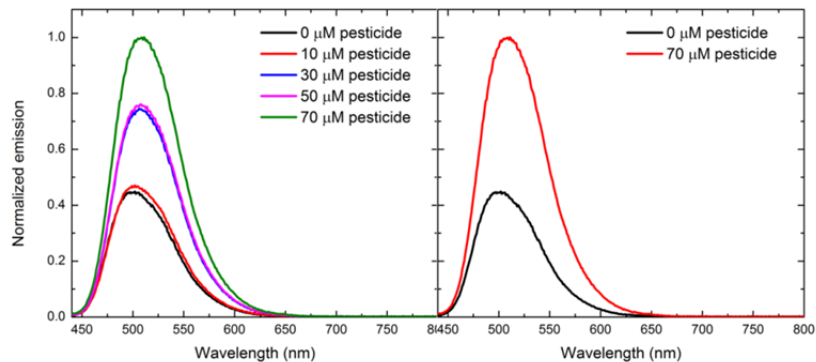
Polymer 11



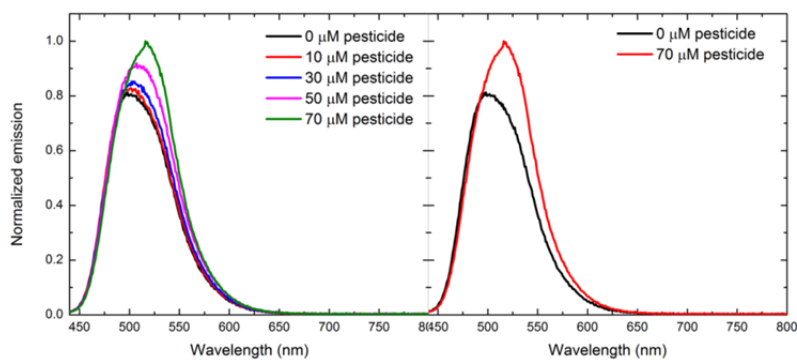
SUMMARY FIGURES FOR FLUORESCENCE EXPERIMENTS

[Polymer] = 1.25 E-3 M; NANOPARTICLE SOLUTIONS

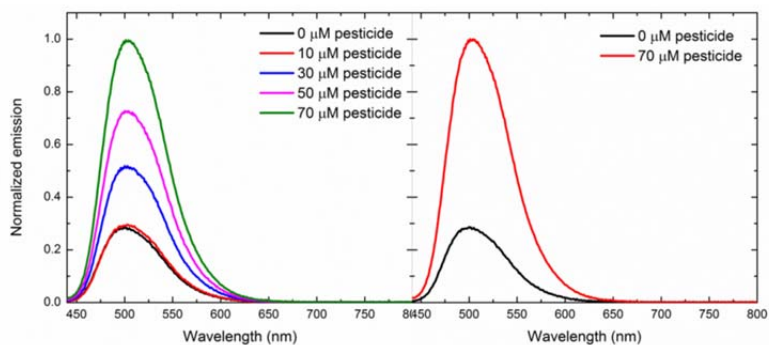
Analyte 1; Polymer 7



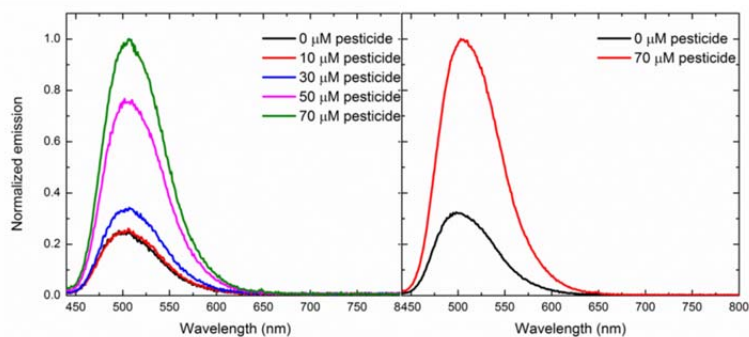
Analyte 2; Polymer 7



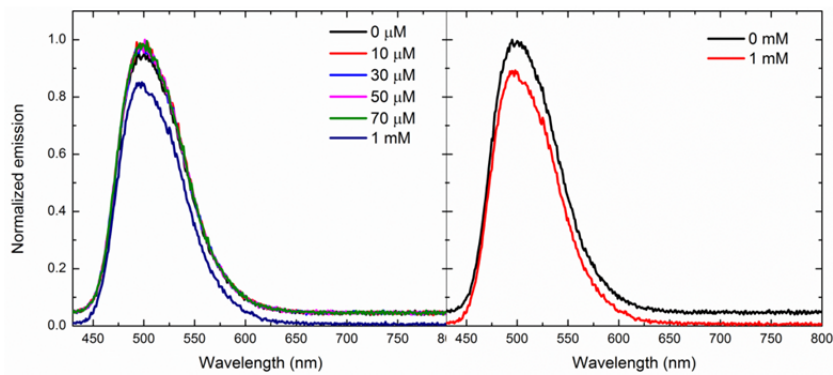
Analyte 3; Polymer 7



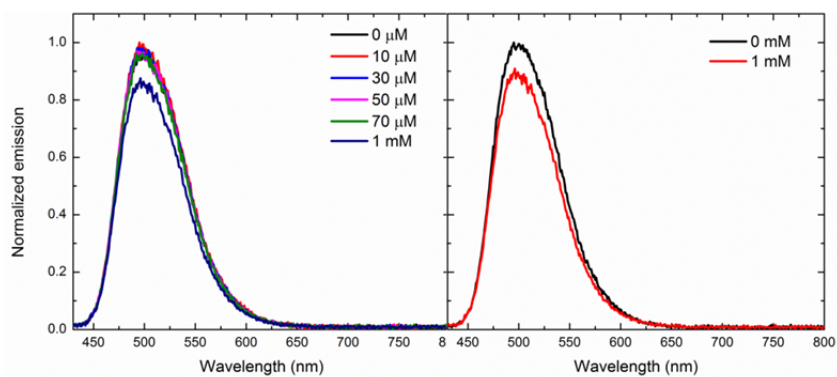
Analyte 4; Polymer 7



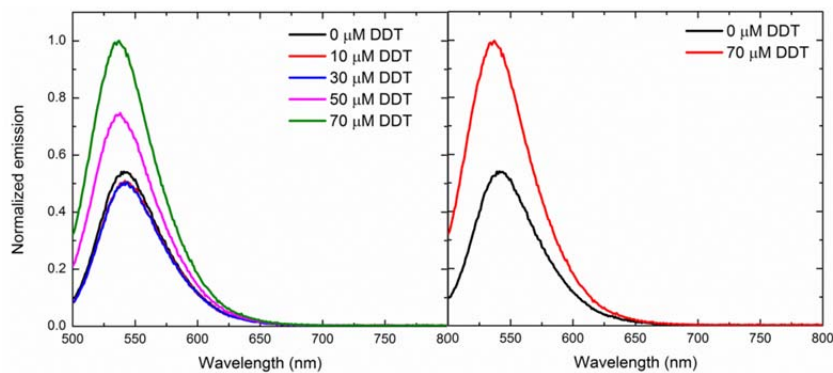
Control Analyte 5; Polymer 7



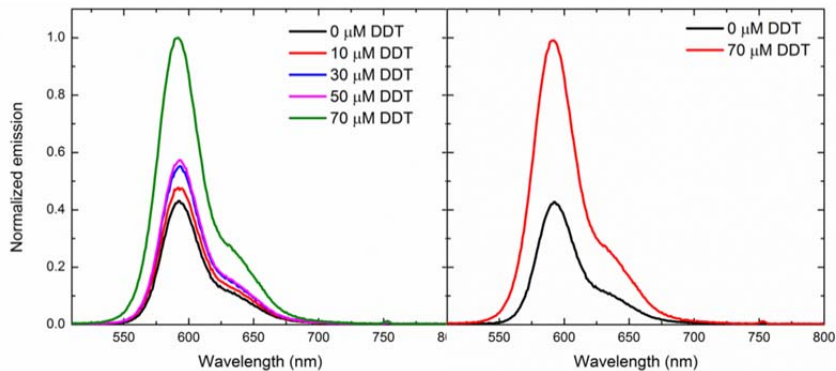
Control Analyte 6; Polymer 7



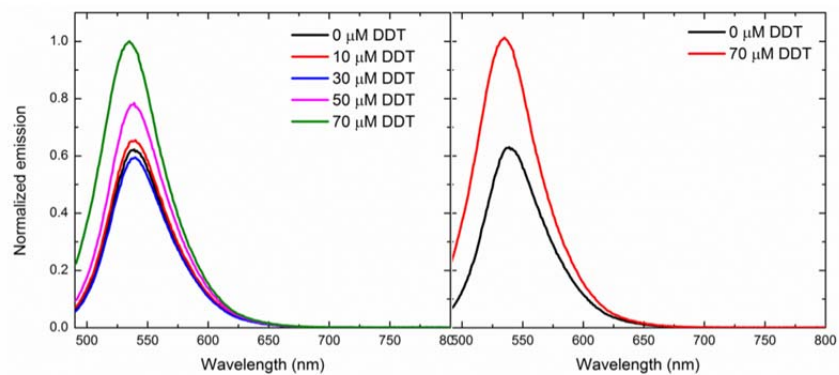
Analyte 1; Polymer 8



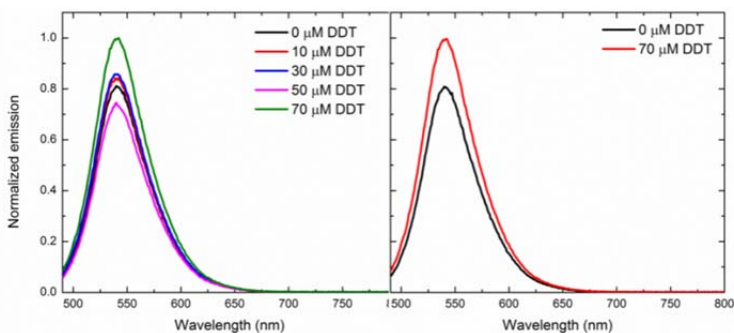
Analyte 1; Polymer 9



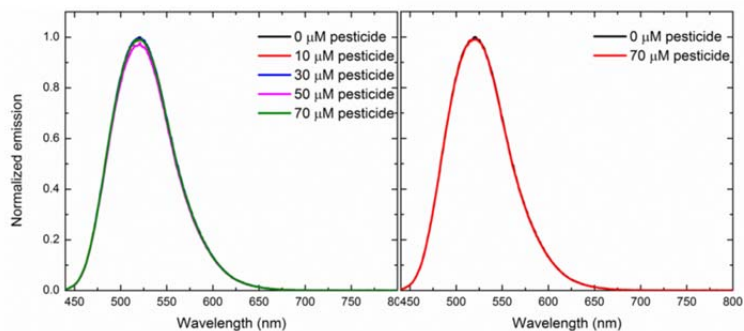
Analyte 1; Polymer 10



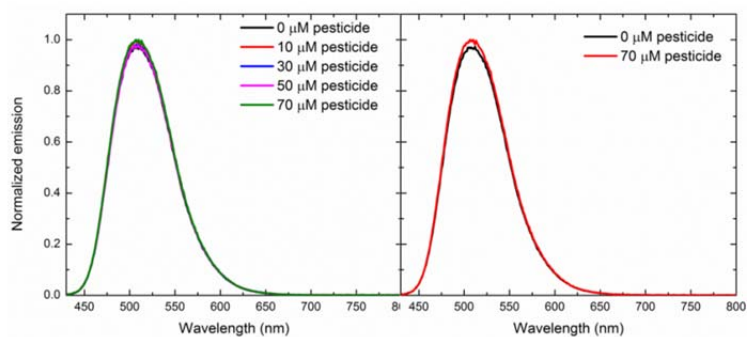
Analyte 1; Polymer 11



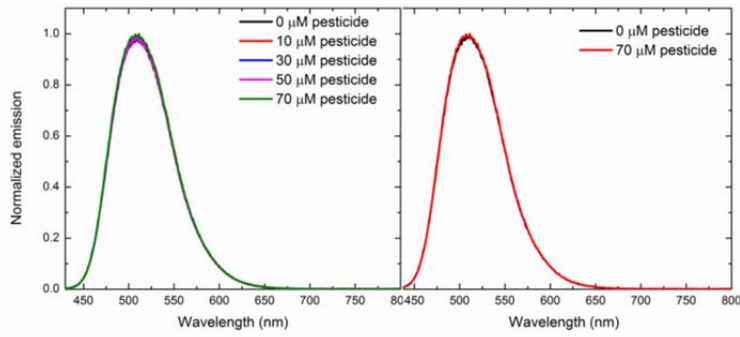
[Polymer] = 1.25 E-3 M; FREE POLYMER SOLUTIONS
Analyte 1; Polymer 7



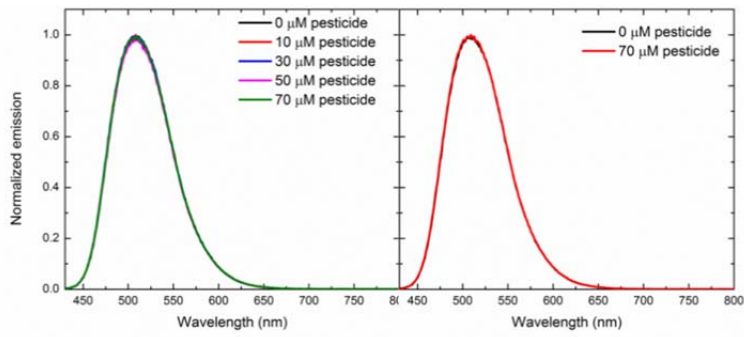
Analyte 2; Polymer 7



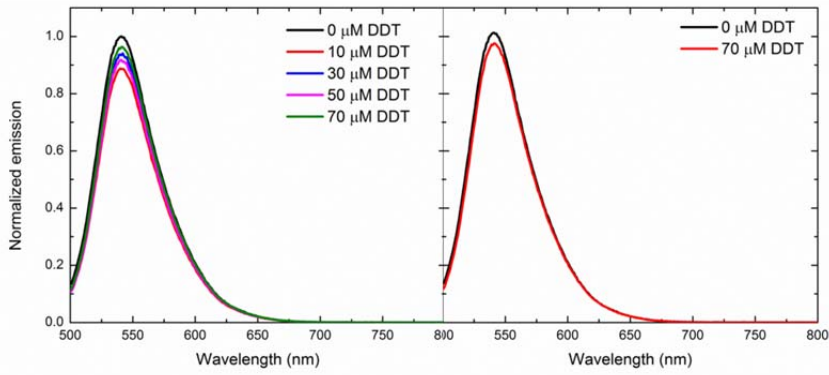
Analyte 3; Polymer 7



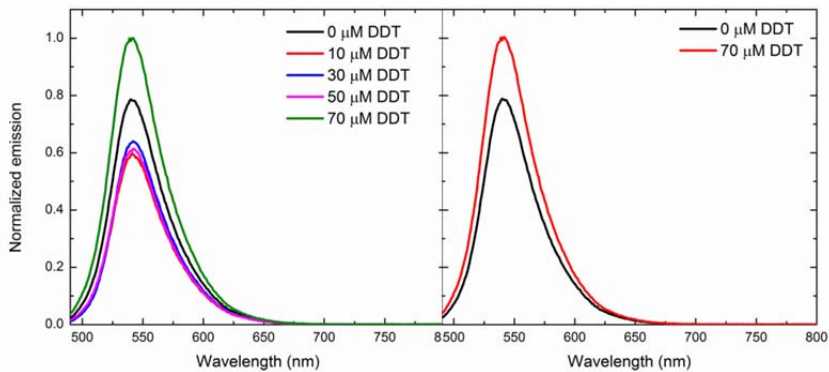
Analyte 4; Polymer 7



Analyte 1; Polymer 8

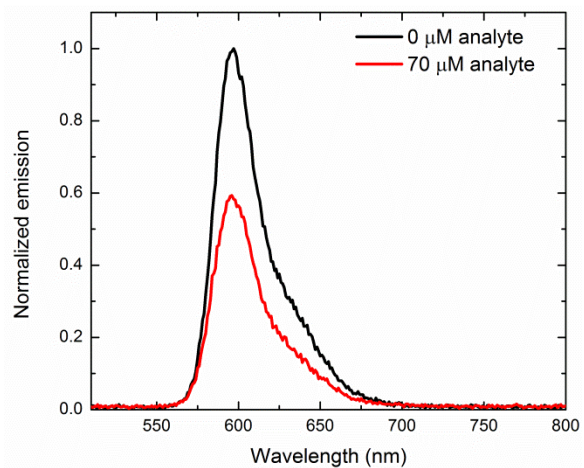


Analyte 1; Polymer 10

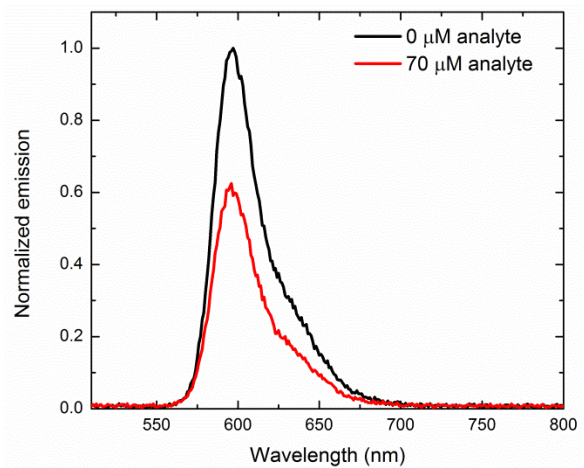


SUMMARY FIGURES FOR CONTROL ANALYTES

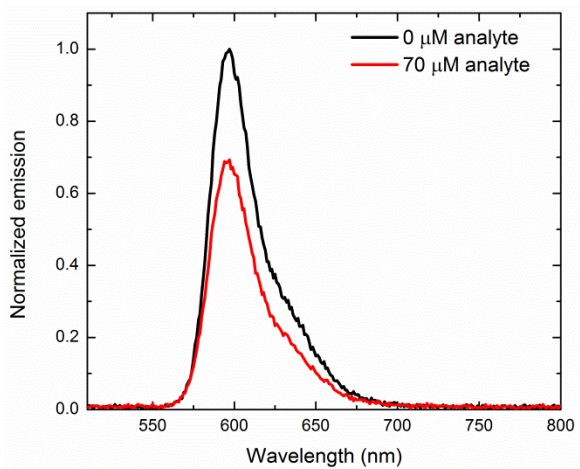
Bisphenol A (BPA)



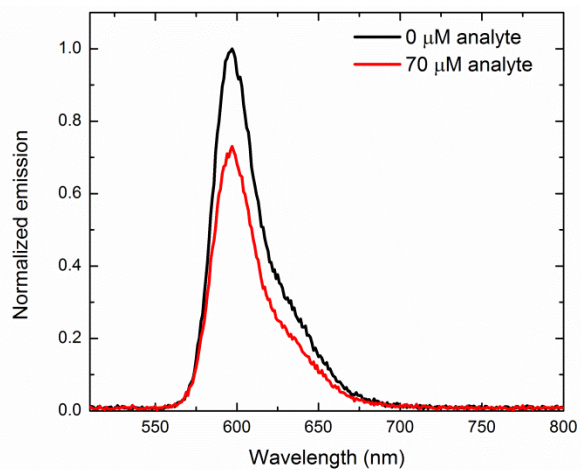
o-Dichlorobenzene



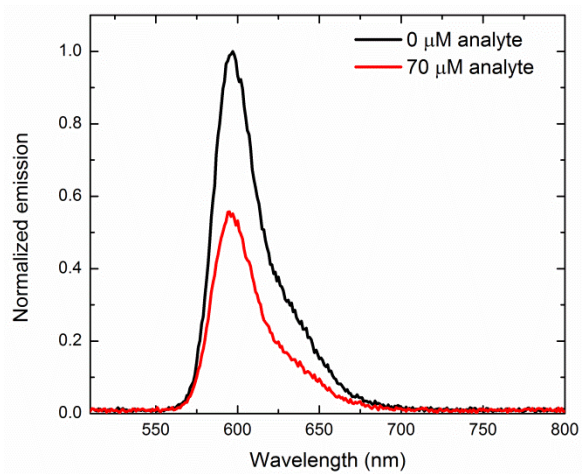
Diphenylmethane



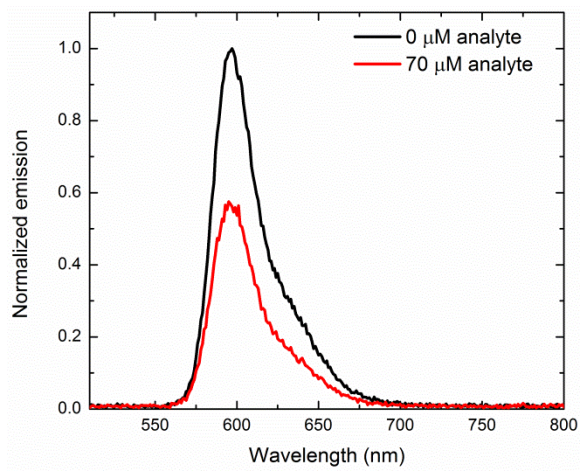
1,1-Diphenylpropane



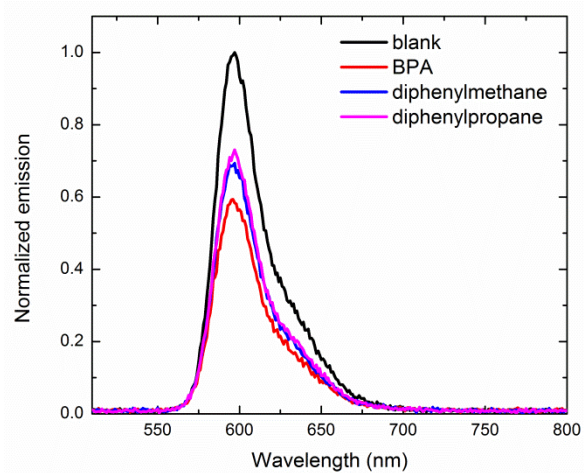
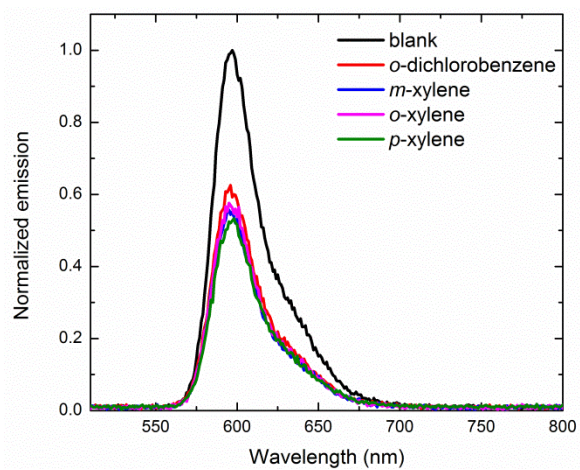
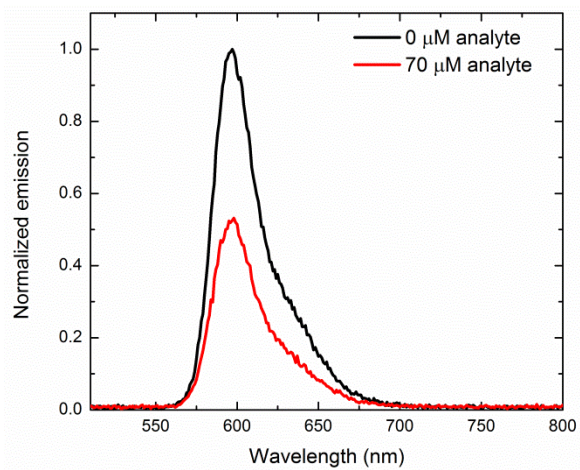
m-Xylene



o-Xylene



p-Xylene



REFERENCES

¹ Brouwer, A. M. *Chemistry International -- Newsmagazine for IUPAC* **2011**, 33 (6).